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REPORT

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Abstract

Denitrification was measured in the Bunnefjord in the Inner Oslofjord using a ^{15}N technique. High denitrification rates were found in a density gradient in the oxic-anoxic interface. Maximum *in situ* denitrification rate was 1.5 mmol$N \cdot m^{-3} d^{-1}$ at 70m depth. Integrated *in situ* denitrification rate was estimated to 13 mmol $N \cdot m^{-2} d^{-1}$ for the water column.

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Denitrification in the water column of an intermittently anoxic fjord

Preface

This report is the result of project P-966035 ”Etablering og bruk av ^{15}N-metode for måling av denitrifisering i det marine miljø” funded as part of NIVA`s institute programme ”Stoffomsetning, retensjon og transport av N, P og C gjennom estuarier og fjorder til kystvann”. Svein Kristiansen, Department of Biology, University of Oslo, was assigned to lead the work scientifically and practically. The undersigned project leader contributed primarily as discussion partner, fieldwork assistant and co-author in preparation of the present report. Unni Efraimsen was in charge of practical arrangements and all titrations of oxygen-samples from the O_2/H_2S boundary layer during the field work in Bunnefjorden on board F/F Trygve Braarud, UiO. The ^{15}N-samples were analyzed during our visit at University of Aarhus, Aarhus, Denmark. We greatly appreciate the help there received from dr. Lars Peter Nielsen and his colleagues at Department of Microbial Ecology.

Oslo, 31.05.99

Morten Schaanning

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Summary

Denitrification was studied in the water column in the Bunnefjord, inner part of the Oslofjord in southern Norway, during September - October 1998. The study also included an evaluation of available methods, and we decided to use a ^{15}N-technique (the isotope pairing method). The fjord is 150 m deep and during the field study it was anoxic below ca. 80 m depth. A pronounced NO_2 peak was found in O_2-poor water around 70 m depth, and the maximum NO_2 concentration was 4.4 mmol $^{-3}$ at 74 m. The concentration of NO_3 drastically decreased with the depth, and both NO_3 and NO_2 were depleted in the anoxic deep water.

Denitrification was measured in the surface (4 m) and at several depths in the O_2-poor deep water (66 - 100 m). The samples were incubated for 1, 2, 4, 10 and 20 days. No significant denitrification rate was found in the surface and a pronounced lag phase of 4 - 10 days was found in the samples from all other depths. High *in situ* denitrification rates were found in the oxic-anoxic interface, and the maximum rate was 1.5 mmol N m^{-3} d^{-1} at 70 m depth. A significant linear correlation between the *in situ* denitrification rate and the ambient nitrate concentration indicated that the *in situ* rate was regulated by available nitrate in the oxic-anoxic interface. It is suggested that *in situ* denitrification was confined to a density gradient around 70 m depth in the oxic-anoxic interface and that integrated *in situ* denitrification rate was 13 mmol N m^{-2} d^{-1}.

Denitrification rates in the oxic-anoxic interface in the Bunnefjord were very high and denitrification was a major N sink in the fjord.

1. Introduction

1.1 Background and objectives

Nitrogen is a bioessential element in proteins of living organisms as well as in numerous organic and inorganic compounds present in the environment. The biogeochemical cycle of nitrogen is complex and incompletely understood. Crucial processes such as nitrogen fixation and denitrification control the exchange between the major atmospheric reservoir (N_2-gas) and the biological nitrogen cycle. Denitrification is the least understood process and it is frequently used to balance nitrogen-cycles (Jaffe, 1992).

Several studies have adressed denitrification at chemoclines in shallow, marine sediments. Few studies have, however, adressed similar chemical environments when occurring in stagnant water masses. Deep water in the Bunnefjord (inner part of the Oslofjord) regularly turns anoxic, and nutrient data from the fjord indicate that denitrification is an important process affecting nitrogen distribution in the fjord water. The present investigation was initiated to assess the significance of denitrification as a natural process removing bioavailable nitrogen from the fjord environment. The main objective of this pilot study was to evaluate, establish and apply a feasible method to measure denitrification at oxic-anoxic boundaries in the water column.

1.2 Theory on denitrification

Several reviews of denitrification are available (see Hattori 1983; Koike and Sørensen 1988; Seitzinger 1988; Zumft 1992; Yoshinari and Koike 1994). Only aspects important for denitrification in fjord waters will briefly be discussed below.

Payne (1973) defines denitrification as reduction of NO_3 to elemental nitrogen by a series of anaerobic respiratory processes:

$$NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_20 \rightarrow N_2$$

The processes are carried out by many facultative anaerobic bacteria. These bacteria can also grow in water rich in O_2 where they use O_2 for respiration (Payne 1973). Denitrification is controlled by several factors including O_2 , organic carbon, temperature and supply of NO_3. In many fjords the concentration of oxygen varies from saturation in the surface water to anoxic conditions in the deep water. The effect of O_2 on denitrification is not fully understood, and it is usually assumed that the process occurs under anoxic and near-anoxic conditions (Payne 1981; Robertson and Kuenen 1990). Rönner and Sörensson (1985) found in Baltic deep water that denitrification occurred only if the O_2 concentration was less than 0.2 ml^{-1}. Other results show, however, that denitrification may occur also at higher concentrations of O_2 , especially if easily assimilable carbon compounds and/or detritus are abundant (see Rheinheimer 1992).

Denitrification has been identified as a major nitrogen sink in the overall N budgets of many aquatic ecosystems (Seitzinger et al. 1993). It is of special interest in coastal and estuarine areas receiving high nutrient loads because denitrification may prevent excessive eutrophication by removing NO_3 as N_2 (Seitzinger 1988; Mantoura et al. 1991). Using a mass balance approach, substantial denitrification rates have been estimated for the Bunnefjord (Nygaard and Bjerkeng 1992) and other Norwegian fjords (Stigebrandt and Aure 1988; Aure and Danielssen 1998). To our knowledge, the rates presented here are the first directly measured denitrification rates from a Norwegian fjord.

1.3 The isotope pairing method

Several methods have been used to quantify denitrification (Seitzinger 1993; Yoshinari and Koike 1994). Seitzinger et al. (1993) compared 3 of the most commonly used methods in aquatic sediments: acetylene inhibition, ^{15}N tracer and N_2 flux. They demonstrated that the acetylene inhibition method underestimated denitrification in an aquatic sediment, especially under NO_3-poor conditions. Results from method tests in sediments do not necessarily apply for the water column. We are, however, not aware of any similar comparison from the water column. After discussions with colleagues at the University of Aarhus, we decided to use an improved ^{15}N technique called the isotope pairing method (Nielsen 1992 and 1994).

A more detailed description of the experiments and the calculations are given in appendix D. In principle, aliquots of ^{15}N (usually as $^{15}NO_3$) are added to a series of subsamples of the water sample in which denitrification is to be determined. The samples are then incubated at *in situ* temperatures for a time series of 0-20 days. Nitrogen gas will be produced during the incubation, and discrete peaks of $^{14}N^{14}N$, $^{14}N^{15}N$ and $^{15}N^{15}N$ are determined on an isotope ratio mass spectrometer. The denitrification rates are then calculated from the measured production of $^{14}N^{15}N$ and $^{15}N^{15}N$ and from the frequencies of $^{15}NO_3$ and $^{14}NO_3$ used during the denitrification. The method has mostly been used in sediments, though it may be applied in almost any aquatic environment.

Precautions have to be taken when using the method. The most important is to ensure an uniform mixing of the added ^{15}N. This may be a major problem in sediments, but should be a minor problem in water samples. The added $^{15}NO_3$ should not affect denitrification of the natural $^{14}NO_3$. Ideally, denitrification from natural $^{14}NO_3$ is independent of added $^{15}NO_3$ (Nielsen 1994). This may be checked by running experiments with different amounts of $^{15}NO_3$ added. We did not do such experiments here.

2. Material and methods

2.1 Sampling

All samples were collected and shipboard analyses performed at the hydrographic station (EP1) in the Bunnefjord in the inner Oslofjord. Water depth at the station was ca. 150 m. Hydrography, nutrients and O_2 conditions at the station are well known from monitoring surveys (Magnusson et al. 1997). Deep water in the Bunnefjord regularly turns anoxic. It was last renewed in 1996, and during our surveys in 1998, the deep water was anoxic below 80 - 90 m.

The samples were collected using 1.7-liter Niskin bottles mounted on a Neil Brown CTD on 4 dates (21, 25 and 29 September and 20 October 1998). Most of the samples were collected near the oxic-anoxic interface between 60 and 90 m. Denitrification was measured at 10 depths on 25 September (4, 66, 70, 72, 74, 76, 78, 82, 86 and 100 m) and at 4 depths on 25 September (70, 72, 74 and 82 m). Main sampling depths were determined *ad hoc* based on analyses of NO_2 in water samples collected immediately after arrival at the station.

2.2 Analytical

Temperature and salinity were measured and sigma-t was calculated using a Neil Brown CTD. The buoyancy frequency (the Brunt-Väisälä frequency) is often used to estimate the strength of vertical density gradients in the ocean (Phillips 1966) . To more accurately identify weak density gradients, the buoyancy frequency was estimated:

$$N^2 = - (g/\rho)(d\rho/dz)$$

where N (rad s^{-1}) is the buoyancy frequency, g is the gravitational acceleration (= 9.82 m s^{-2}), ρ is a reference density (=1000 kg m^{-3}) and z (m) is the depth. The period (τ, unit s) of the wave can be estimated from the frequency: $\tau = 2\pi N^{-1}$.

Oxygen and H_2S concentrations were measured immediately after sampling using Winkler titration and backtitration with thiosulphate according to Andersen and Føyn (1969). Ammonium and NO_2 concentrations were measured 1 h after sampling according to Strickland and Parsons (1972) and Koroleff (1976) using small sample volumes (10 ml). The other nutrient samples (NO_3, PO_4, $Si(OH)_4$, total N and total P) were conserved with sulphuric acid or chloroform and analysed using standard methods for seawater analyses (NIVA, 1999). The NO_3 concentrations given below are corrected for any NO_2.

The samples for denitrification measurements were incubated in triplicate using 6.7 ml tubes (Exetainer). The tubes were carefully filled as described for BOD bottles in Strickland and Parsons (1972) to avoid O_2 and N_2 contaminants from the air. The isotope was quickly added (25 µl 1.0 mM 99 at-% $^{15}NO_3$) and the tubes were immediately capped. The tubes were incubated in the dark at *in situ* temperatures (15 ^oC for the 4 m sample and 7 ^oC for all other samples) for 1, 2, 4, 10 (11) and 20 days. The incubations were terminated by carefully adding 50 µl formaldehyde (37 % solution) with a syringe trough the septum. Care was taken to avoid air bubbles when adding the formaldehyde.

Head-space gas was analyzed at the University of Aarhus using an isotope ratio mass spectrometer (Sira Series II, VG Isotech, Middlewich, Chesire, U.K.). Excess $^{14}N^{15}N$ and $^{15}N^{15}N$ (corrected for natural abundances) were measured and denitrification rates were calculated as shown in Nielsen

(1992). The concentration of N_2 in the water was calculated from temperature and salinity (Riley and Skirrow 1975). The procedure is described in detail in appendix E.

A pronounced lag phase of 4 - 10 days preceded nitrogen production in all experiments. The denitrification rates presented below are based on the production rates for the period between day 4 and day 10 or 11.

An additional experiment was conducted to test if anoxic ammonium oxidation (Anammox) occurred in the NO_2 maximum. Selected samples were treated with surpluses of $^{15}NH_4$ (50 µl 5.0 mM 99 at-% $^{15}NH_4$) and $^{14}NO_2$ (50µl 1.0 mM $^{14}NO_2$). The procedure is described in detail in appendix E.

3. Results and discussion

3.1 Hydrography, oxygen and nutrients

Surface temperature decreased from 15 to 8 ^{o}C from the first cruise on 21 September until the forth cruise on 20 October. The temperature below 50 m did not change much between the cruises (< 0.1 ^{o}C). It did, however, decrease from 7.7 ^{o}C at 50 m to 7.0 ^{o}C at 90 m. The main pycnocline was situated between 5 and 15 m (Fig. 1a). Most of our samples were collected between 60 and 90 m (near the oxic-anoxic interface), and the density profiles from all 4 cruises were similar in this depth interval (Fig 1b).

Two density gradients were identified in the deep water, one at 40 - 55 m and one around 70 m. The latter was weak, but the plots of the buoyancy frequency (Figs. 1c and 1d) showed that the gradient remained present at 63 - 78 m throughout the sampling period (21 September to 20 October). Characteristic buoyancy frequencies and periods in the upper ocean are in the order 0.01 rad s^{-1} or 10 min (Mann and Lazier 1991). Vertical density gradients will be much stronger in freshwater influenced coastal areas. The very high buoyancy frequencies in the main pycnocline (maximum 0.13 rad s^{-1} at 13 m, values not shown) are typical in such areas (Lund-Hansen et al. 1994).

Figure 2 shows complete depth profiles of NO_3, NO_2, NH_4 and O_2 collected during the first cruise (21 September). Most of the samples during the following 3 cruises were collected between 60 and 90 m, and all observations within this depth interval are plotted in Fig. 3.

Nitrate and O_2 was depleted below 70 - 75 m and NO_2 peaked within the same depth interval. Back-titration with thiosulfate clearly showed the presence of H_2S in water samples collected at 90 m and below. However, Ammonium concentration was high (3 - 8 mmol m^{-3}) above the pycnocline and in the O_2-poor watermass below 80 m (Appendix A).

The steep concentration gradients of the various compounds were clearly confined to the deep density gradient (63-78m depth). At its upper boundary oxygen decreased from >0.7 ml l^{-1} at 60 m depth to 0.1-0.3 ml l^{-1} at 70 m. Below this depth, the concentration of O_2 decreased slowly with increasing depth. Disregarding two anomalous high concentrations of 0.28 ml l^{-1}, a slight drop from ca 0.15 ml l^{-1} to 0.05 ml l^{-1} appeared to occurr below 78 m whereas negative values (H_2S) were confined to depths below 88m. Odour from H_2S was noticed in samples from below 80 m, and H_2S may have been the primary electron donor responsible for the final depletion of O_2 somewhere between 78 and 88m.

Immediately below the major depletion of O_2 at about 70 m, concentrations of nitrate decreased to non-detectable values at 76-78 m. Nitrite peaked within the same depth interval (70-76m) yielding maximum concentrations up to 4.39 mmol m^{-3} at 74 m depth. Ammonium was rarely detectable in water samples with high concentrations of NO_2, but increased sharply below 78m depth, corresponding to the lower boundary of the deep density gradient.

Narrow NO_2 concentration maxima were frequently observed close to 70 m depth. Both the maximum concentration and the depth at which it was found apparently changed between the cruises (Table 1). During the cruises on 29 September and 20 October, water samples were collected at one meter intervals between 68 and 78 m. The NO_2 peaks were found within 1 - 2 meter of the water column and the NO_2 concentration increased up to 6 times within 1 m (from 0.59 mmol m^{-3} at 71 m to 3.51 mmol m^{-3} at 72 m on 29 September, Fig. 4). Our sampling procedure was not designed to sample such gradients, and the variable NO_2 concentration in the NO_2 maximum was probably caused by

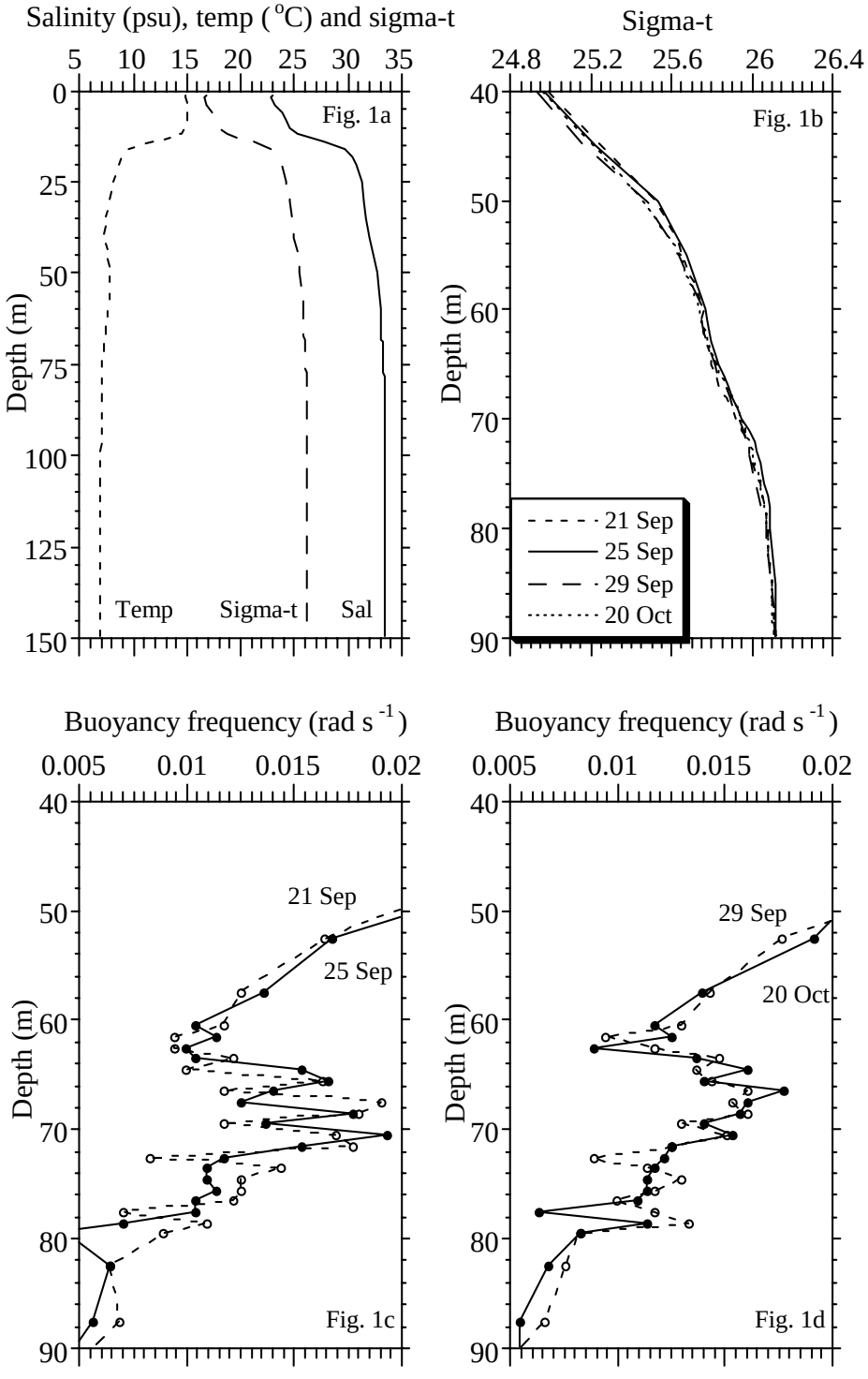

Figure 1. Hydrography in the Bunnefjord in September - October 1998. Depth profiles of temperature (°C), salinity (psu) and sigma-t on 25 September (Fig. 1a) and sigma-t between 40 and 90 m from all the 4 cruises (21, 25 and 29 September and 20 October 1998; Fig. 1b). Buoyancy frequencies between 40 and 90 m are shown for each cruise in Fig. 1c and 1d (open symbols represent first date).

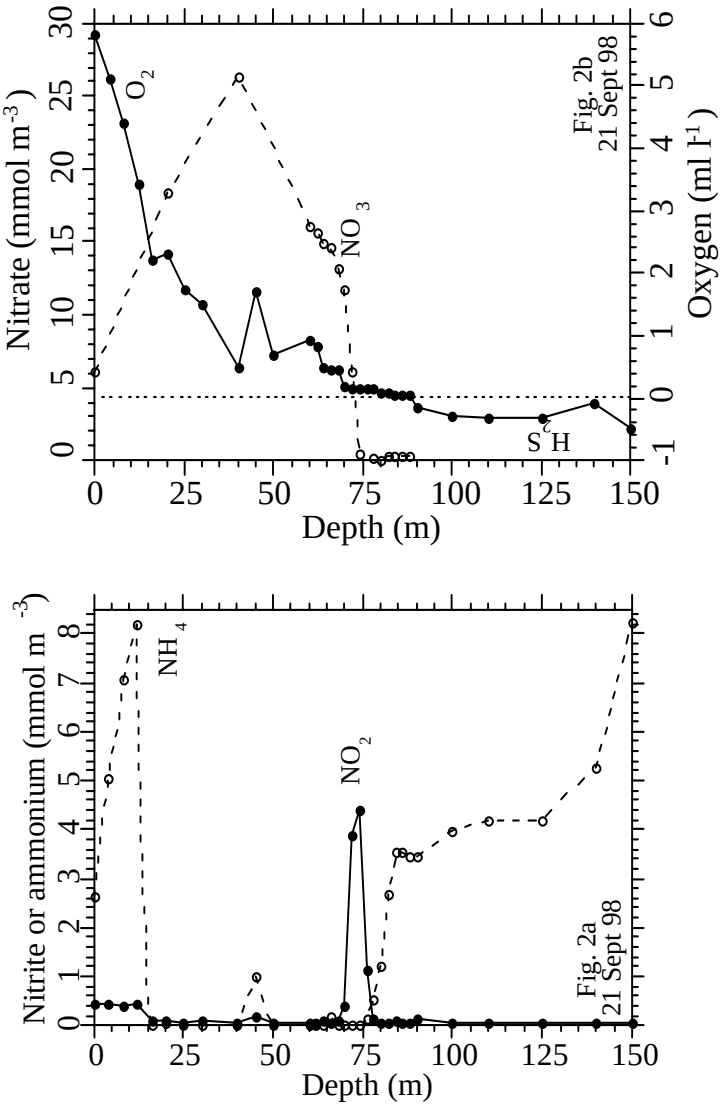

Figure 2. Depth profiles of nutrients, O_2 and H_2S on 21 September 1998. Concentrations of NH_4 and NO_2 in mmol m^{-3} (Fig. 2a) and concentrations of NO_3 in mmol m^{-3} and O_2 and H_2S in ml l^{-1} (Fig. 2b). Hydrogen sulphide concentration is plotted as negative O_2 concentration, and the broken line is zero O_2 concentration.

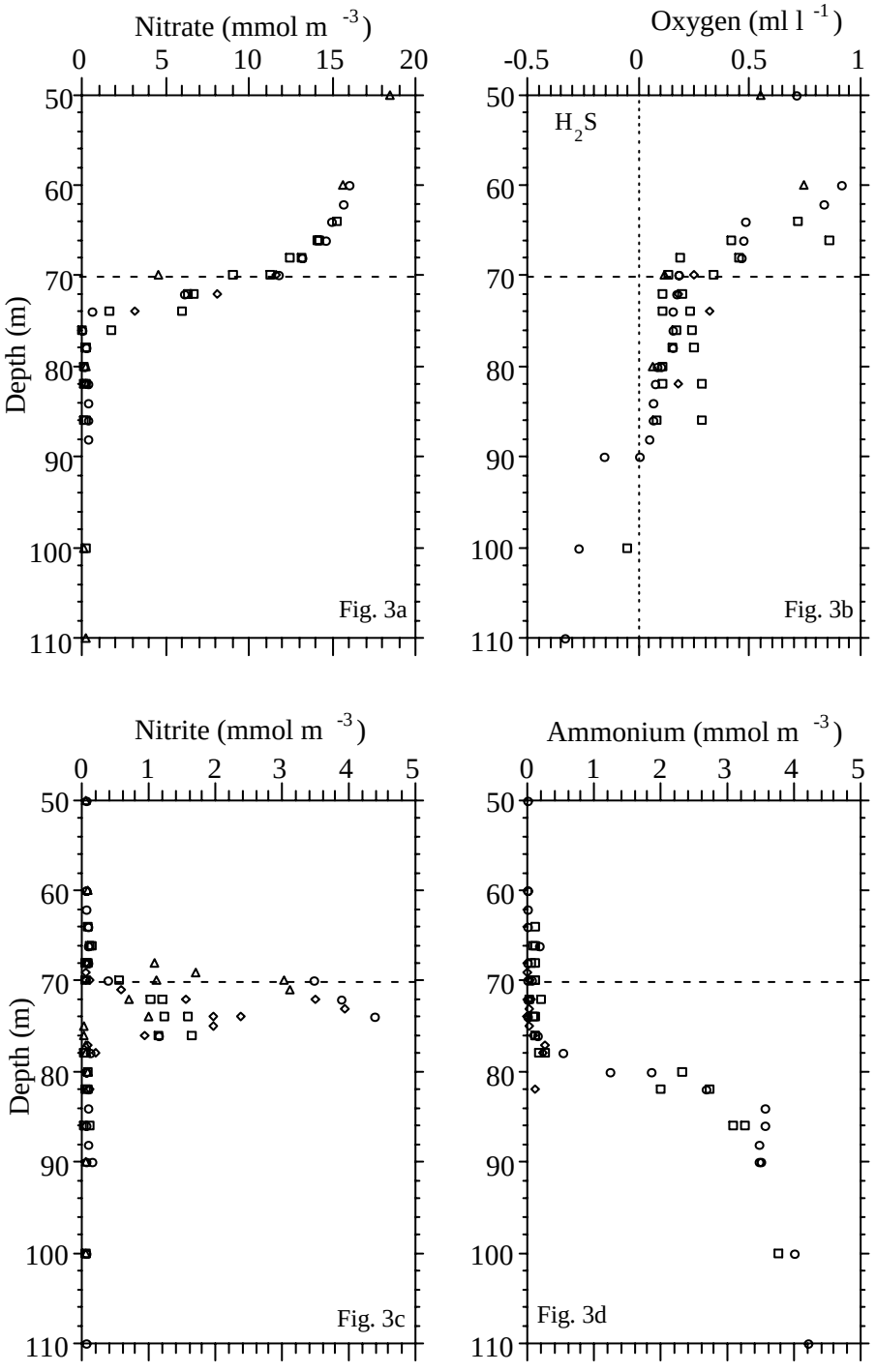

Figure 3. Concentrations of nutrients, O_2 and H_2S between 50 and 110 m from all the 4 cruises. Concentrations of NO_3 in mmol m^{-3} (Fig. 3a), of O_2 and H_2S in ml l^{-1} (Fig. 3b) and of NO_2 and NH_4 in mmol m^{-3} (Figs. 3c and 3d respectively). Hydrogen sulphide concentration is plotted as negative O_2 concentration. The broken lines are zero O_2 concentration (vertical) and 70 m (horizontal). Circles, squares, diamonds and triangles are concentrations from 21, 25, 29 September and 20 October respectively.

inappropriate sampling equipment (1.7 liter Niskin bottles mounted on a CTD).

The depth of the NO_2 maximum was apparently 1 -2 m higher up in the water column on 20 October than on 29 September. Simultaneously, however, a corresponding change of salinity also occurred. Salinity is a conservative variable, and using salinity as reference, the NO_2 peaks from the 2 dates fitted reasonably well (Fig. 4). The observed changes in salinity may have been caused by internal waves. According to Gade (1967) internal waves probably exist in the inner part of the Oslofjord.

3.2 Nitrogen deficit in the deep water

The concentration of NO_3 decreased from ca 15 mmol m^{-3} at 64 m depth to the detection limit at 76 m. Even though the concentrations of NO_2 and NH_4 increased in different parts of the deep water, the sum NO_2 + NH_4 never exceeded 5 mmol m^{-3} (Fig. 3). Thus, the increase of nitrate and ammonium could not account for the decrease in the NO_3 concentration. Furthermore, stoichiometric models for decomposition of organic matter (Redfield 1958 and other) predict a coupling between the increase of N-, P- and Si-nutrient species. The concentrations of Tot P, PO_4 and $Si(OH)_4$ increased with depth (Fig. 5) whereas the concentration of Tot N showed a major decrease between 40 and 80 m. Finally, Fig. 5d showed a general decrease of the N:P ratio with depth. Thus, the vertical profiles determined on 20 October consistently showed a specific loss of nitrogen in the deep water. The classical N:P ratio in unpolluted water is 16 (Redfield 1958) , and low N:P ratios in anoxic deep water has been attributed to denitrification (Seitzinger 1988) .

The difference Tot P - PO_4 is called undefined P (Undef P) and the difference Tot N - NO_3 - NO_2 is called undefined N (Undef N) below. Undef P mainly consists of particulate P and dissolved organic P. Undef N mainly consists of particulate N, NH_4 and dissolved organic N. Ammonium was only measured in a few of the samples from 20 October and NH_4 is therefore included in Undef N. The concentration of Undef P was low in all the samples and most of Tot P in the deep water was PO_4 (Fig. 5). The concentration of Undef N was high in all the samples, and the concentrations > 10 mmol m^{-3} close to the surface and below 80 m were caused by high NH_4 concentrations. High NH_4 concentrations (4 -8 mmol m^{-3}) were found both close to the surface and below 80 m during the previous cruises (Figs 2 and 3 and Appendix A). The few NH_4 measurements from 20 October also showed high concentrations close to the surface and below 80 m (3 - 13 mmol m^{-3}, values not shown).

Concentrations of NO_3, NO_2, NH_4 and O_2 are plotted against each other in Fig. 6. Disregarding one exceptionally high concentration of NO_2 (1.2 mmol l^{-1}) observed within the main pycnocline on 20 October, the figure shows that the peak concentrations of NO_2 (> 0.65 mmol m^{-3}) occurred in water containing < 0.3 ml l^{-1} of O_2 and < 0.2 mmol m^{-3} of NH_4, but up to 8.1 mmol l^{-1} of NO_3.

Table 1. Concentrations in the NO_2 maximum during the four cruises. Depth (m), NO_2 concentrations (mmol m^{-3}) and salinity (psu).

Date	**21 Sept**	**25 Sept**	**29 Sept**	**20 Oct**
Depth (m)	74	76	73	71
NO_2 (mmol m^{-3})	4.39	1.65	3.95	3.12
Salinity (psu)	33.242	33.299	33.214	33.207

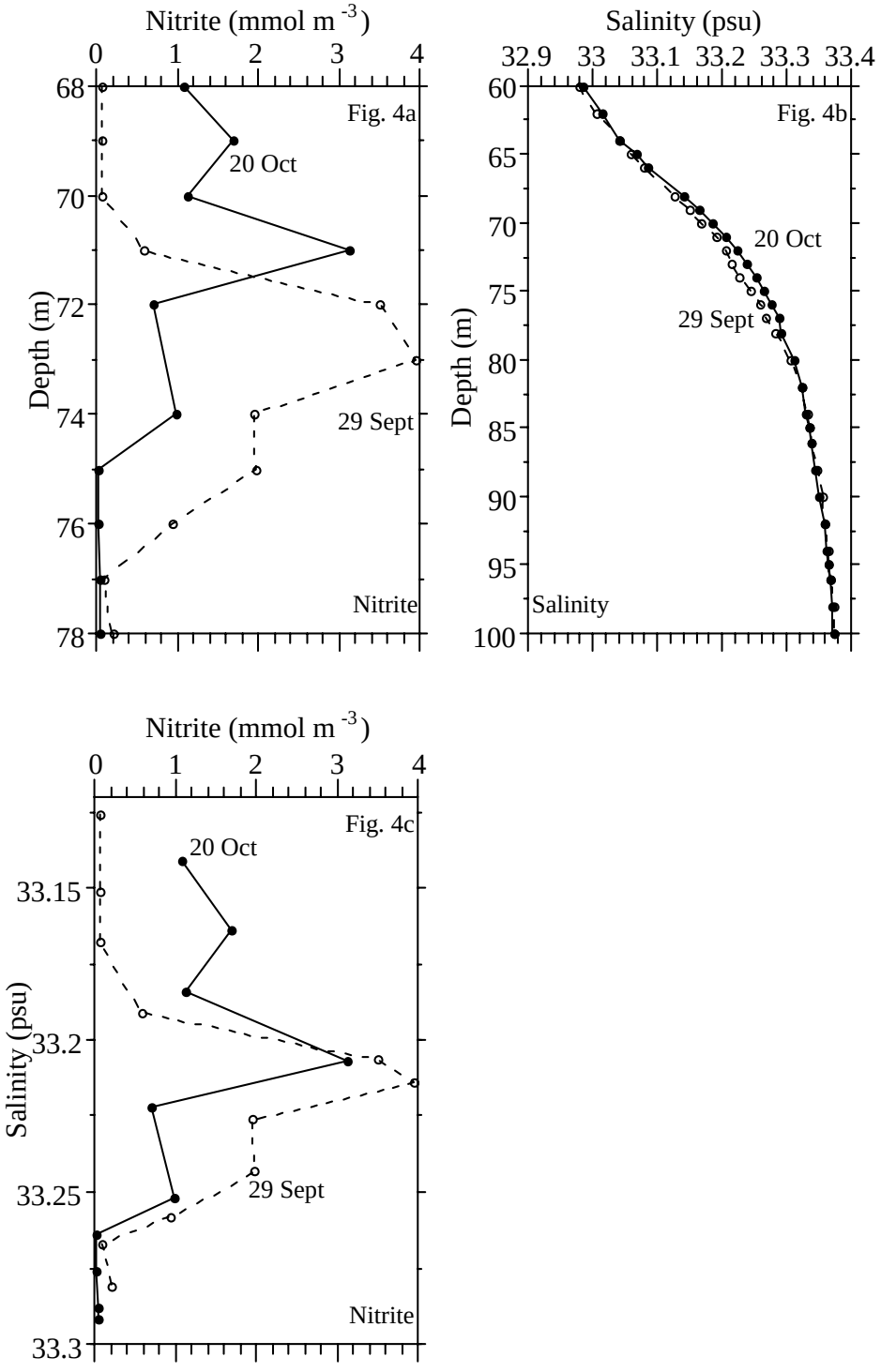

Figure 4. Depth profiles of NO_2 concentration (mmol m^{-3}; Fig. 4a) and salinity (psu; Fig. 4b) and "depth profile" of NO_2 concentration plotted against salinity (psu; Fig. 4c) from 29 September and 20 October.

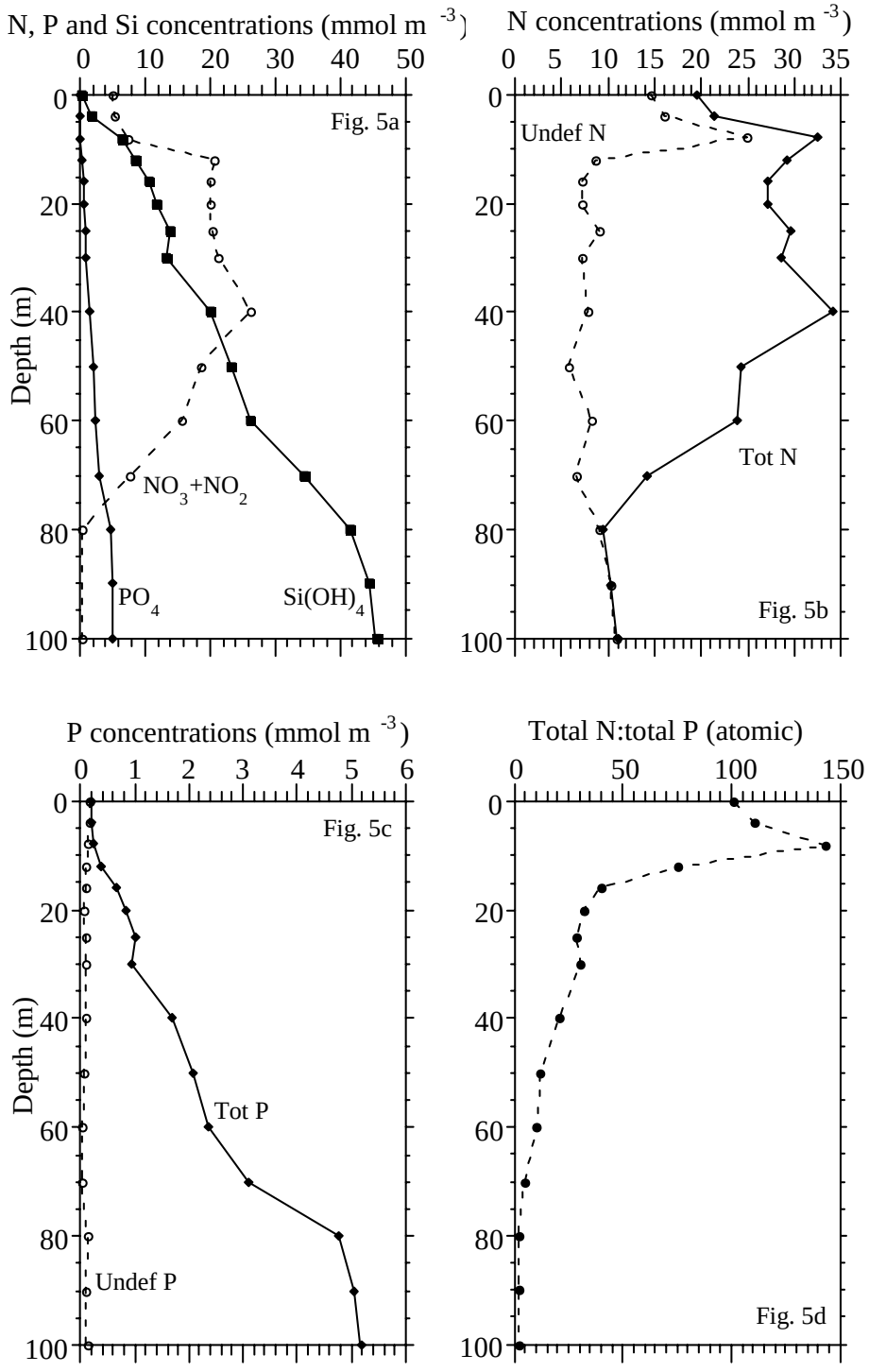

Figure 5. Depth profiles of N-, P- and Si-compounds on 20 October. Undefined N = Tot N - NO_3 - NO_2. Undefined P = Tot P - PO_4. The ratio total N: total P (atomic) are given in Fig. 5d. All the concentrations are in mmol m^{-3}. Data from 20 October. NH_4 not measured.

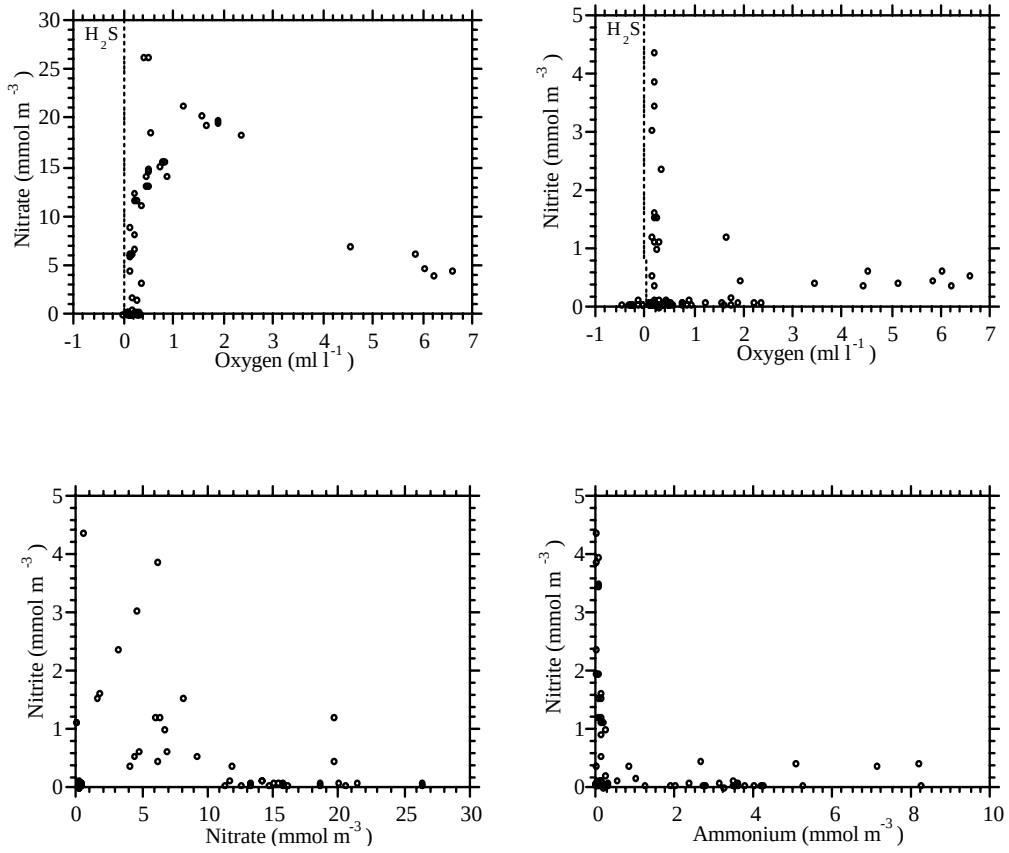

Figure 6. Concentrations of NO_3, NO_2 and NH_4 (mmol m^{-3}) and O_2 and H_2S (ml l^{-1}) plotted against each other. Hydrogen sulphide concentration is plotted as negative O_2 concentration, and the dotted lines are zero O_2 concentration.

3.3 Denitrification

3.3.1 Labelled nitrogen production in incubation tubes

Denitrification was measured in samples from 10 depths on 5 September and from 4 depths on 29 September. Similar experiments have previously not been conducted in the Oslofjord or in any other Norwegian fjord. Time series experiments were therefore performed with all the samples by incubating subsamples for 1, 2, 4, 10 (or 11) and 20 days.

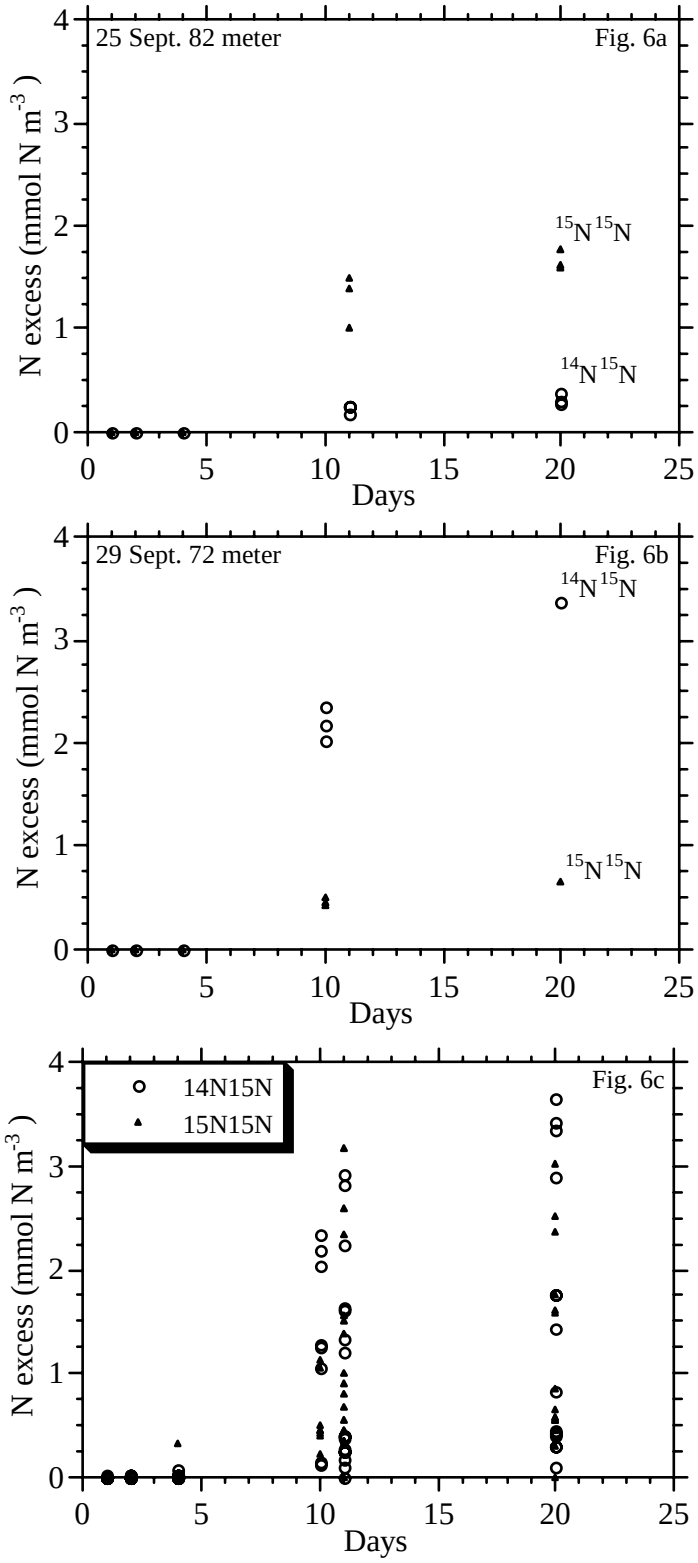

Figure 7. $^{14}N^{15}N$ excess and $^{15}N^{15}N$ excess in mmol N m^{-3} plotted against incubation time in days in samples from 82 m on 25 September (Fig. 7a) and from 72 m on 29 September (Fig. 7b). Values from all the time series experiments are summarized in Fig. 7c. The ambient concentrations of NO_3 and NO_2 were 0.2 and 0.05 mmol m^{-3} respectively in the sample from 25 September and 8.1 and 1.54 mmol m^{-3} respectively in the sample from 29 September.

Two characteristic time series experiments are shown in Figs. 7a,b together with a plot showing results from all 14 experiments. Fig. 7a shows NO_3-depleted water from 82 m depth (0.2 mmol NO_3 m^{-3}) and 7b shows NO_3-rich water from 72 m depth (8.1 mmol NO_3 m^{-3}). As consequences of the ambient NO_3 concentration, the single-labelled N_2 ($^{14}N^{15}N$) was low in the NO_3-depleted sample and high in the NO_3-rich sample. The double labelled N_2 ($^{15}N^{15}N$) showed the opposite pattern, it was high in the NO_3-depleted sample and low in the NO_3-rich sample.The experiments also showed that in many samples incubated for 10 days or more, most of the added $^{15}NO_3$ (usually > 90%) was recovered as labelled nitrogen gas ($^{14}N^{15}N$ or $^{15}N^{15}N$). No doubt, in these samples NO_3 had been converted to N_2 gas. This showed that denitrification had been the dominating nitrogen transformation process. Little or no denitrification was, however, observed in samples incubated for 4 days or less.

3.3.2 The lag phase

The lag phase of 4 - 10 days was consistent in all samples (Fig. 7 and Appendix D), and it was therefore probably caused by a common factor.

Lag phases and long incubation times (days - weeks) seem to be obligatory in water column denitrification experiments (Goering and Dugdale 1966; Goering 1968; Rönner and Sörensson 1985; Brettar and Rheinheimer 1991) . Somewhat shorter incubation times (hours - days) are usually used when working in sediments (Rysgaard et al. 1993; Seitzinger et al. 1993; Nielsen 1994) .

Denitrification is assumed to be controlled by factors like O_2, temperature and the supply of NO_3 and organic matter. The O_2 concentration was low in most of the samples (usually < 0.3 ml l^{-1}). The lag phase was pronounced also in samples smelling of H_2S and no detectable O_2 (100 m on 25 September) and it appears unlikely that all samples could have been "contaminated" with O_2 during handling (see methods). All incubation tubes were carefully checked for bubbles and only 9 out of 234 tubes were rejected because of small bubbles. The samples were incubated at *in situ* temperature which was about 15ºC for the 4 m sample and 7ºC for all the other samples. 4 mmol m^{-3} of $^{15}NO_3$ were added to all samples. It is unlikely that insufficient organic matter stopped denitrification for 4 - 10 days and then ample organic matter suddenly was available in all tubes and the denitrification rates increased rapidly. Thus, neither O_2, temperature or supply of NO_3 or organic matter probably caused the lag phase.

Denitrification may, however, occur via successive reduction to intermediate products such as NO_2, NO, N_2O (see section 3). Some of the intermediates produced during denitrification may accumulate (Betlach and Tiedje 1981) and thereby delay production of N_2. This accumulation may occur because some bacteria reduce NO_3 to N_2 while other only reduce NO_3 to NO_2 (Payne 1973) . Nitrite may also accumulate because NO_3 partly represses the enzyme nitrite reductase (Fenchel and Blackburn 1979) . The high concentrations of NO_2 in the deep boundary (Fig. 3c) layer confirmed the significance of reduction to NO_2 as a separate step preceding further reduction to N_2. If a pool of ^{15}N labelled intermediates produced from the added $^{15}NO_3$ had to precede any formation of labelled nitrogen gas, a lag phase would have to appear in our results, even though the overall denitrification process had proceeded at a steady rate throughout the incubation period.

An additional experiment was conducted in December to test whether the lag phase resulted from intermediate NO_2 production (see Lag phase experiment in Appendix D). Similar lag phases were found however, also after addition of $^{15}NO_2$ and NO_3 did apparently not influence the reduction of NO_2. It was concluded from this experiment that if the lag phase was a result of a multi-step reduction from NO_3 to N_2, NO_2 was not the only intermediate involved.

Lag-phases might alternatively be explained by exponential growth (cell doubling) of an initially small population of bacteria. One obvious common factor was the addition of NO_3 to all incubation tubes. If the addition of (labelled) nitrate triggered the growth of an initially small or inactive population of denitrifying bacteria, the rates calculated from labelled N_2 production would not be representative for the *in situ* process. However, lag phases were found also in samples in which significant amounts of nitrate was present before the nitrate addition (Fig. 7b).

We did not monitor the bacterial community during our experiments, neither when the samples were collected in the fjord nor during the experiments. The time series of N excess in Fig. 7 did not fit well with a simple exponential function. N excess rather seemed to increase step-wise with a maximum production 4-10 days after incubation.

At the fjord station, the decrease with depth of NO_3 and Tot N and the increase of PO_4 and $Si(OH)_4$ (Figs. 2, 3 and 5), clearly showed that nitrogen was lost from the water column. Because samples were drawn at 2 m depth intervals in the relevant part of the water column, it appears likely that at least some of our samples must have had a viable population of denitrification bacteria when incubated. Since lag phases were observed in all samples, exponential growth of initially small bacteria populations can hardly be the common factor explaining the lag phase observed in our experiments.

Another alternative explanation might be an initial inhibition of the bacteria due to some unknown factor imposed during transplantation from in situ to the test tube environment. Both confinement of the samples in tubes (or other reaction vessels; Ferguson et al. 1984) and the walls of the tubes may seriously affect the bacterial community during incubation and thereby change the denitrification rates. The tubes and the stoppers have been thoroughly tested and found ideal for denitrification experiments (L.P. Nielsen, personal communication). Working with sediments in incubation chambers, Rysgaard et al. (1993) found stable denitrification rates for at least 4.5 days. However, water column bacteria may be more sensitive to transplantation than sediment bacteria and the complete set of equipment and reagents have not been tested for inhibiting factors.

The variable rate of labelled N2-production over the incubation period raised the question of which period to use in rate calculations. The denitrification rates given below are based on the 4-10 (or 11) days incubation period.

3.3.3 Denitrification at the main chemocline

In Fig. 8, the *in situ* (D_{14}, see methods) and potential ($D_{14} + D_{15}$) denitrification rates are plotted next to the ambient concentrations of NO_3, NO_2, NH_4 and O_2. All the rates are also given in Appendix C. The highest *in situ* (0.4 - 1.5 mmol N m^{-3} d^{-1}) and potential denitrification rates (0.8 - 2.1 mmol N m^{-3} d^{-1}) were found at 66 - 74 m. The *in situ* rate was low below 74 m while the potential rate increased again below 78 m. Oxygen and NO_3 concentrations were important factors in regulating the denitrification rates (Fig. 9). Oxygen concentration was high in the only sample collected in the surface (4 m depth on 25 September), and no significant denitrification was found in the surface sample even after 20 days incubation (Appendix B). The O_2 concentration was < 0.9 ml l^{-1} in the remaining samples, and the denitrification rates were apparently regulated by the ambient NO_3 concentration. It was significant linear correlations between the denitrification rates and ambient NO_3 concentrations ($p < 0.001$, Fig. 9). It was no significant correlations between the denitrification rates and ambient NO_2 concentration ($p > 0.5$) or between the denitrification rates and ambient O_2 concentration ($p > 0.5$). The highest NO_2 concentrations (1.57 and 2.39 mmol m^{-3} at 74 m on 24 and 29 September respectively) were found 2 - 4 m below the corresponding high denitrification rates (Fig. 8). Apparently, the NO_2 concentration and the denitrification peaks were vertically separated 2-4 m with the denitrification peak on top of the NO_2 concentration peak. All samples from the same depth

and date in Fig. 8 were withdrawn from the same water bottle, and the vertical separation between the NO_2 concentration and the denitrification peaks cannot have been caused by sampling error.

Few and variable denitrification rates are published from the water column in marine ecosystems (Rönner and Sörensson 1985; Seitzinger 1988; Brettar and Rheinheimer 1991; Nygaard and Bjerkeng 1992). The published measured rates (acetylene inhibition and ^{15}N) in O_2-poor water were in the range 0.04 - 18 mmol N m^{-3} d^{-1}, and the highest rates were found using ^{15}N techniques. Our *in situ* and potential rates from 66 - 74 m (0.4 - 2.1 mmol N m^{-3} d^{-1}) are within the range of the rates reported. Bjerkeng (NIVA, unpublished) has estimated loss rates (denitrification) in the Bunnefjord using a mass balance approach (Nygaard and Bjerkeng 1992). His highest rates were 0.06 - 0.2 mmol N m^{-3} d^{-1} which is 1 order of magnitude lower than our measured rates.

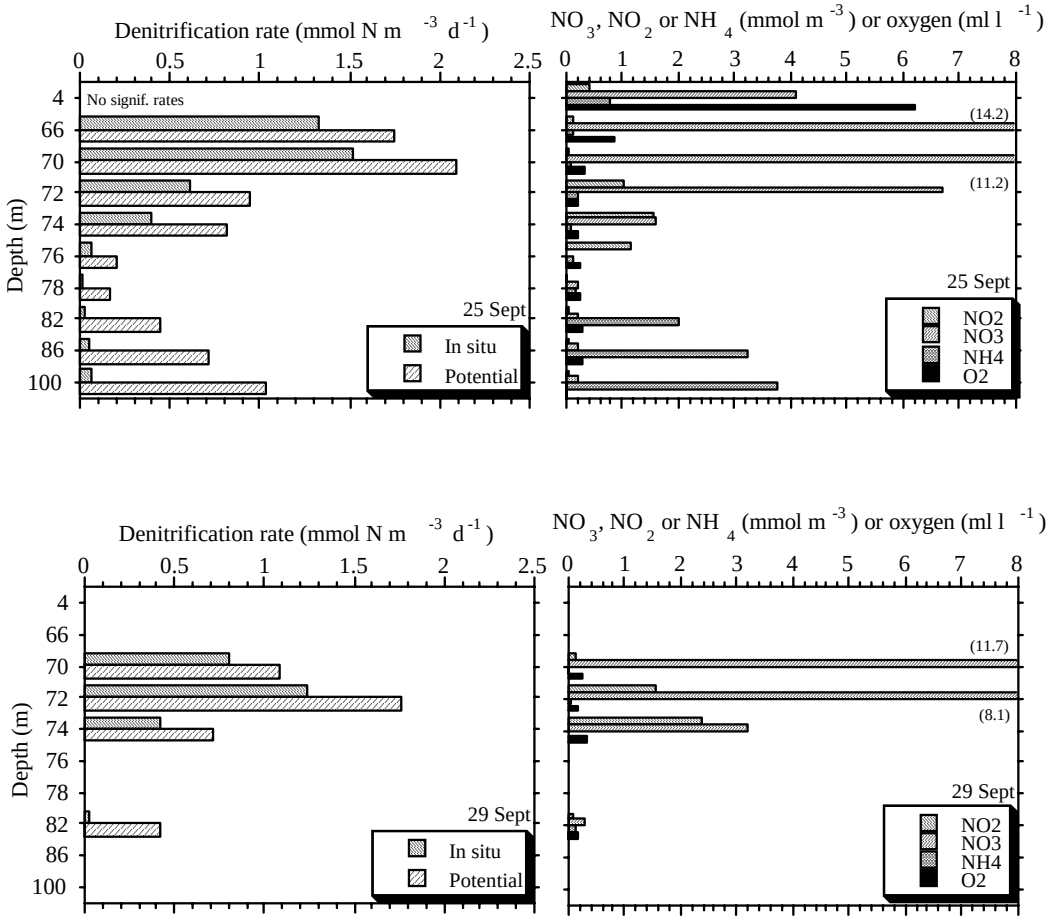

Figure 8. *In situ* and potential denitrification rates (mmol N m^{-3} d^{-1}) on 25 and 29 September in the 2 left figures and concentrations of NO_2, NO_3, NH_4 (mmol m^{-3}) and O_2 (ml l^{-1}) in the 2 right figures. Four high NO_3 concentrations are off scale and are given in parentheses. No significant denitrification rates were measured in sample from 4 m on 25 September.

The highest denitrification rates were found at 66 - 74 m (Fig. 8) and 40 - 93 % of the ambient NO_3 and NO_2 in the samples (added ^{15}N not included) were denitrified during the first 10 - 11 days (Table 2). Assuming constant denitrification rate, all ambient NO_3 and NO_2 in the samples (added ^{15}N not included) would have been denitrified within 11 - 19 days. Both ambient NO_3 and NO_2 concentrations and the *in situ* denitrification rate were very low below 76 m. The corresponding values in Table 2 may be biased and values are therefore given in parenthesis.

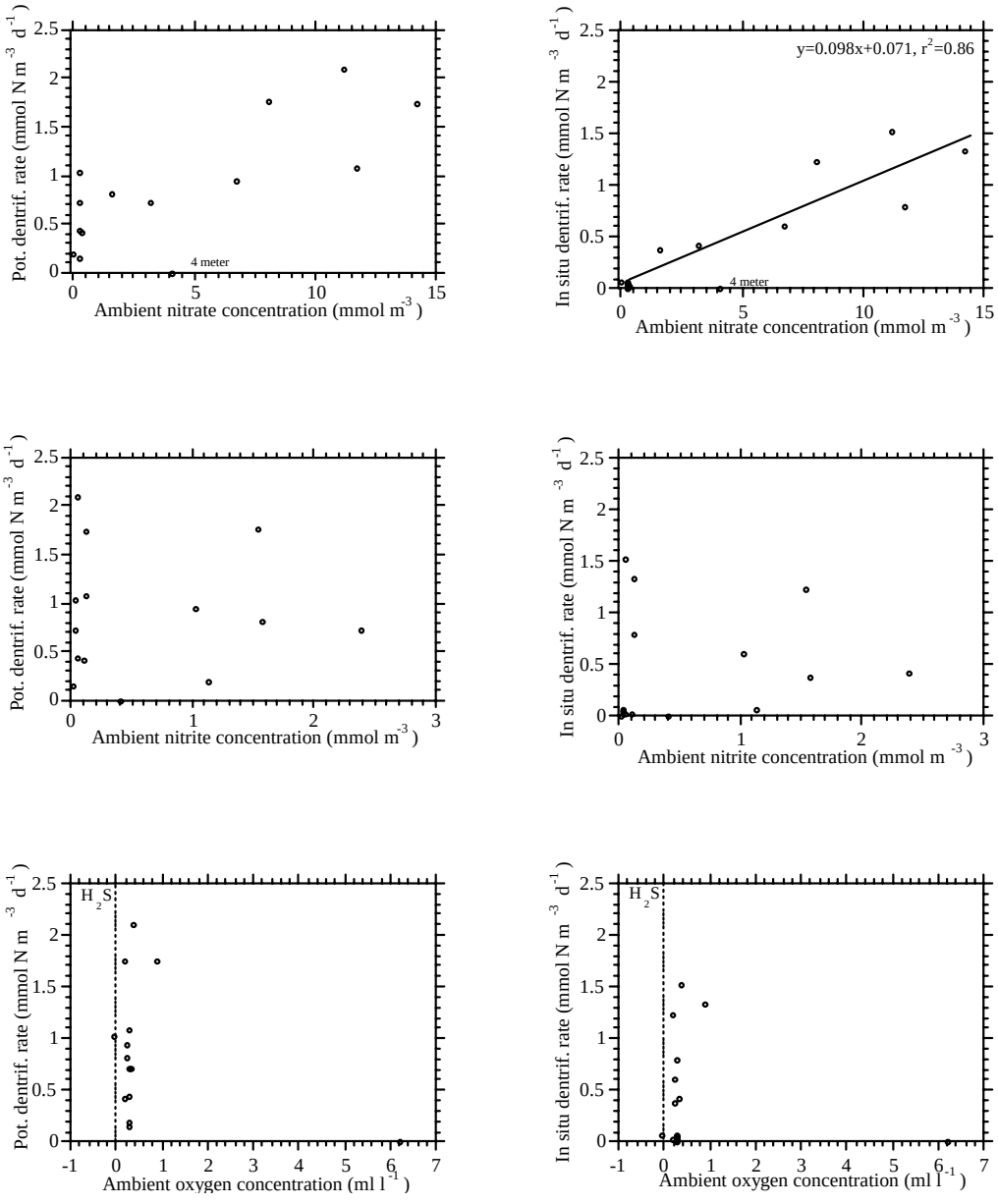

Figure 9. Potential and *in situ* denitrification rates (mmol N m^{-3} d^{-1}) plotted against ambient concentrations of NO_3, NO_2, NH_4 (mmol m^{-3}) and O_2 (ml l^{-1}). Hydrogen sulphide concentration is plotted as negative O_2 concentration. The full line is linear regression of *in situ* denitrification rate versus ambient NO_3 concentration, omitting the 4 m value. The dotted lines are zero O_2 concentration.

Table 2. Amount (%) of ambient NO_3 and NO_2 denitrified after 10 days and number of days to depletion of ambient NO_3 and NO_2 by denitrification. The values in parenthesis are based on low concentrations and rates (see the text).

Date/Depth	% denitrified after 10 days	Days to depletion
25 September		
4	0	-
66	65	15
70	93	11
72	55	17
74	84	12
76	34	23
78	(21)	(25)
82	(64)	(11)
86	(106)	(8)
100	(127)	(8)
29 September		
70	40	19
72	76	12
74	44	17
82	(38)	(16)

Denitrification was not measured between 4 m with no significant denitrification rate and 66 m with high rates. If one assumes that NO_3 alone limits the *in situ* denitrification rate, the rate can easily be estimated from the detailed NO_3 profile collected on 21 September and the equation given in Fig. 9. This works reasonable well below 66 m (Fig. 10a and b). Oxygen will, however, stop denitrification (Fig. 9), and somewhere between 66 m and 4 m the denitrification rate ceased because of increased O_2 concentration (Figs. 2 and 3). Four estimates of the integrated *in situ* denitrification rate are therefore given in Table 3. Estimate 1 (9.6 mmol N m^{-2} d^{-1}) is based on the rates modelled from the NO_3 concentrations from 21 September and estimate 2 (10.4 mmol N m^{-2} d^{-1}) is based on the measured rates from 25 September, both from 66 - 100 m. Both estimates probably underestimate the integrated rate because the denitrification rate was high at 66 m. The O_2 concentration was similar at 64, 66 and 68 m (0.5 ml l^{-1}), and denitrification in the 64 - 66 m layer is therefore included in estimate 3 (13.4 mmol N m^{-2} d^{-1}). The O_2 concentration was significantly higher between 62 and 50 m (0.7 - 0.9 ml l^{-1}). Denitrification in the 50 - 66 m layer is nevertheless included in estimate 4 (38.7 mmol m^{-2} d^{-1}). No direct measurements are available to support the increased rates in estimates 3 and 4. It is reasonable that the denitrification rate was similar at 64, 66 and 68 m (see above), and estimate 3 is probably not too high. Estimate 4 is more difficult to evaluate. However, high denitrification rate was found in a sample containing 0.9 ml l^{-1} (66 m on 25 September, Fig. 8). The NO_3 concentration decreased below 40 m while the concentrations of PO_4 and $Si(OH)_4$ gradually increased with depth (Figs. 3 and 5). Low N:P ratios and increased NO_2 concentrations have been used as indications on denitrification (see above). Both occurred below 60 m depth. Consequently, estimate 3 (13 mmol m^{-2} d^{-1}) is the best available estimate of integrated *in situ* denitrification rate at our station. The *in situ* denitrification rates used to calculate estimates 3 and 4 are shown together with the measured *in situ* rates in Fig. 10.

Table 3. Four estimates of integrated *in situ* denitrification rates (mmol N m^{-2} d^{-1}) in the upper 100 m water column. The integrated rates are from different depth intervals and are based on modelled rates, measured rates or on a combination of the two.

Estimate	Depth interval (m)	Integrated *in situ* rate (mmol N m^{-2} d^{-1})
1	66-100 (modelled)	9.6
2	66-100 (measured)	10.4
3	64-100 (measured and modelled)	13.4
4	50-100 (measured and modelled)	38.7[i]

[i]High estimate, see the text.

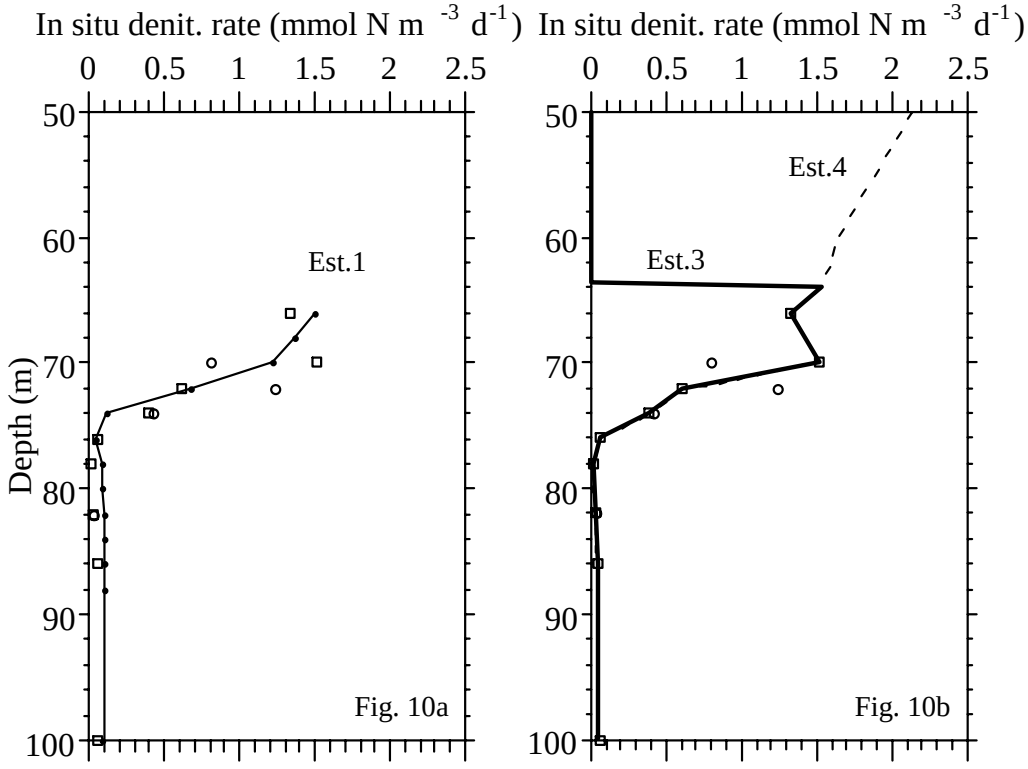

Figure 10. *In situ* denitrification rates (mmol N m^{-3} d^{-1}) from 25 and 29 September (squares and circles respectively). The lines indicate the rates used to calculate estimate 1 (Fig. 10a) and estimates 3 and 4 (solid and broken lines in Fig. 10b) of integrated *in situ* denitrification rates (mmol N m^{-2} d^{-1}). See text.

The hydrography may be of some help to evaluate the different estimates of integrated *in situ* denitrification rate. The highest denitrification rates (66 - 70 m) were measured within the weak density gradient around 70 m (Fig. 1). According to the buoyancy frequency, the deep density gradient was situated at 63 - 72 m on 25 September. Integrated *in situ* denitrification rate in the deep density gradient at 63 - 72 m) was 12.4 mmol m^{-2} d^{-1} which is 93 % of estimate 2 (Tables 3 and 4). The rate below the deep density gradient (72 - 100 m) was 2.5 mmol m^{-2} d^{-1}. The gradients restricted downward transport of nutrients (nitrate) and oxygen, and thereby regulated the *in situ* denitrification rate. Unfortunately, denitrification was not measured in the upper part of the deep density gradient (63 - 65 m) or in the intermediate layer above the deep density gradient. Nevertheless, it is possible that a major part of the denitrification occurred in the deep density gradient and that estimate 3 (13.4 mmol m^{-2} d^{-1}) is a reasonable estimate of the integrated *in situ* denitrification rate.

Table 4. Concentrations and denitrification rates (average ± std. dev.) in the surface layer (4 m), in the deep density gradient (64 - 72 m) and below the deep density gradient (74 - 100 m) on 25 September. Number of samples (n) are given separately for the concentrations of nutrients and oxygen and for the denitrification rates.

Concentrations or rates	**Surface layer (4 m)**	**Deep density gradient (63 - 72 m)**	**Below deep density gradient (74 - 100 m)**
Concentrations			
Nitrite (mmol m^{-3})	0.4	0.4±0.5	0.5±0.7
Nitrate (mmol m^{-3})	4.1	11.4±3.3	0.9±1.7
Ammonium (mmol m^{-3})	0.8	0.1±0.1	1.5±1.5
Oxygen (ml l^{-1})	6.2	0.4±0.3	0.2±0.1
n (for the concentrations)	1	9	12
Denitrification rates			
Potential rate (mmol N m^{-3} d^{-1})	0.0	1.6±0.6	0.6±0.4
In situ rate (mmol N m^{-3} d^{-1})	0.0	1.2±0.5	0.1±0.1
n (for the rates)	1	3	6
Integrated *in situ* rate (mmol N m^{-2} d^{-1})	-	12.4	1.5 (2.5)[i]

[i] 2.5 mmol N m^{-2} d^{-1} in 72 - 100 m.

Denitrification was also measured at a few depths on 29 September, and high rates were again found within the deep density gradient. A deep density gradient was found during all the 4 cruises, though the position of the gradient apparently varied 1 - 2 m within the range 63 - 72 m (Fig. 1). Similar variation (1 - 2 m) was also found in the position of the high NO_2 concentrations around 70 m. By using the salinity as a reference (instead of depth), this variation diminished (Fig. 4). Denitrification was only measured at a few depths at 2 stations and it was no need for using salinity as a reference. When doing a more detailed study of denitrification across density gradients it will probably be necessarily to use salinity as a reference instead of depth. Possibly then, denitrification in the water column at our station was confined to the deep density gradient in the oxic-anoxic interface. The deep density gradient was a weak though stable structure in the water column during all the surveys (Fig. 1). If denitrification really is confined to the deep density gradient, estimates of denitrification will be less laborious:

- It will be easier to choose the "correct" sampling depths.
- Number of direct measurements of denitrification can be reduced.
- Interpolation between direct measurements of denitrification can by greatly improved by including more frequently conducted routine measurements of hydrography (CTD), nutrients (NO_3 and NO_2) and O_2.

It is therefore important to verify if denitrification really is confined to the deep density layer.

The NO_3 concentration decreased below 40 m, though the NO_3 profiles were very similar during the 4 cruises (Fig. 3). At 70 m the concentration of NO_3 decreased significantly with time. It decreased from 11 mmol m^{-3} during the 3 cruises in September to 5 mmol m^{-3} in late October (Fig. 11). The decrease could not be explained by the 1-2 m vertical displacement of the water column (Fig. 4), and it is reasonable to assume that denitrification was the NO_3 sink. Nitrate was only measured at 60, 70 and 80 m on 20 October. The exact magnitude of the NO_3 decrease was therefore uncertain. The high NO_2 concentration at about 70 m (3.12 mmol m^{-3} at 71 m; Table 1) indicated that high denitrification rates still prevailed during the October survey.

Using a mass balance approach, (Stigebrandt and Aure (1988) estimated denitrification from bottom sediments in different fjords on the Norwegian west coast. Their estimated rates were 1 - 2 orders of magnitude lower (0.3 mmol N m^{-2} d^{-1} in deep fjord basins and 0.8 mmol N m^{-2} d^{-1} in shallow fjords). However, Aure and Danielssen (1998) used a similar approach in the outer parts of the Oslofjord and found substantial higher denitrification in the Oslofjord than expected from the estimates above. Using a model, Shaffer and Rönner (1984) found that 80 - 90 % of denitrification in the Baltic occurred in the sediment. However, later direct measurements show that denitrification from the oxic-anoxic interface of the water column and in the sediment may be similar (Brettar and Rheinheimer, 1991). Integrated denitrification rates in the Baltic based on measured rates are in the range 0.2 - 90 mmol N m^{-2} d^{-1} (Rönner and Sörensson 1985; Brettar and Rheinheimer 1991). Our estimates (10 - 14 mmol m^{-2} d^{-1}) are in the lower part of this range.

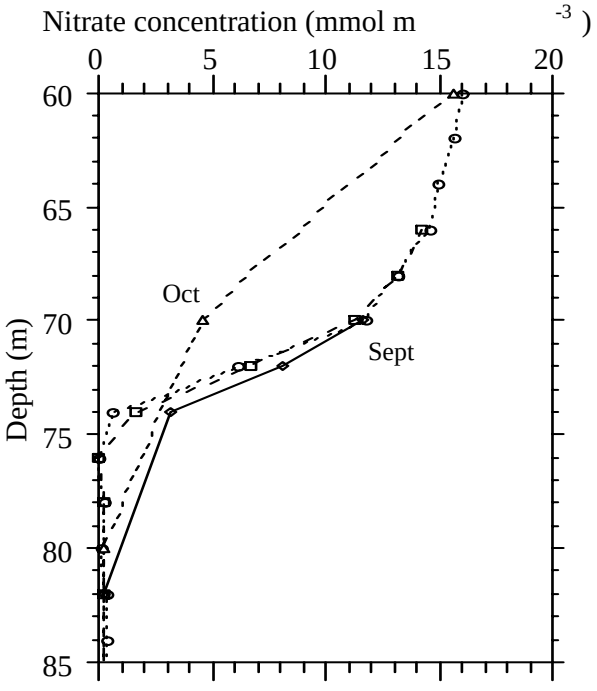

Figure 11. Depth profiles of NO_3 concentration (mmol m^{-3}) between 60 m and 85 m on 21, 25 and 29 September (circles, squares and diamonds respectively) and 20 October (triangles).

3.3.4 The NO_2 peak

The high NO_2 concentrations around 70 m were pronounced during all the cruises (from September through December; Fig. 3 and Fig. D1), and the high concentrations were associated with intense denitrification (Fig. 8).

Increased NO_2 concentration in O_2-poor water has often been associated with denitrification both in coastal regions and in open ocean, and the high concentrations are referred to as the secondary NO_2 maximum (Goering and Cline 1970; Hattori 1983; Codispoti et al. 1986; Lipschultz et al. 1990; Rheinheimer 1992) . A primary NO_2 maximum is situated higher up in the water column, near the bottom of the euphotic zone, and are generated by several other biological processes (Wada and Hattori 1991) . The high concentrations in the oxic-anoxic interface around 70 m (Fig. 3) was probably part of a secondary NO_2 maximum in fjord.

Anderson et al. (1982) modelled NO_2 and NO_3 distributions in oceanic O_2 minimum zones. They found high NO_2 concentrations in O_2 minimum zones. Part of the NO_2 in their study was reduced to N_2 (denitrification) and the rest diffused out of the O_2 minimum zone and was oxidized to NO_3 by nitrifying bacteria. We did not measure nitrification in our samples. Diffusion of NO_2 upwards from the oxic-anoxic interface followed by nitrification may explain the 2 - 4 m vertical separation of NO_2 concentration and denitrification peaks seen in Fig. 8.

3.3.5 Was autumn 1998 part of a typical year?

Anoxic deep water in the Oslofjord may typically be found in the Bunnefjord and in some small local basins (e.g. Bærumsbassenget). Occasionally, the O_2 concentration may be low (around 0.5 ml l^{-1}) also in the more open Vestfjord (western part of the Oslofjord; Magnusson et al. 1997). Most of denitrification in the water column in the Oslofjord probably therefore occurs in the Bunnefjord. The O_2 concentration at 100 m in the Bunnefjord was < 3.1 ml l^{-1} in October during the years 1973 - 1998 (Fig. 12). During these 26 years, O_2 concentration was 0.2 ml l^{-1} in October for 13 years and 1.0 ml l^{-1} in October for 18 years (Table 5). Not surprisingly, the corresponding number of years are somewhat lower at 80 m (12 and 14 years) and somewhat higher at 125 m (13 and 19 years). An O_2 concentration of 0.2 ml l^{-1} has often been used as an upper limit for denitrification. Denitrification may occur at higher O_2 concentrations (see sections 1.2), and we therefore compared 0.2 and 1.0 ml l^{-1} as upper limits for denitrification in Table 5. Irrespective of which upper limit used, denitrification probably occurred in the water column for more than one half of the years. Based on this evaluation, denitrification in the water column should generally be an important N sink in the Bunnefjord.

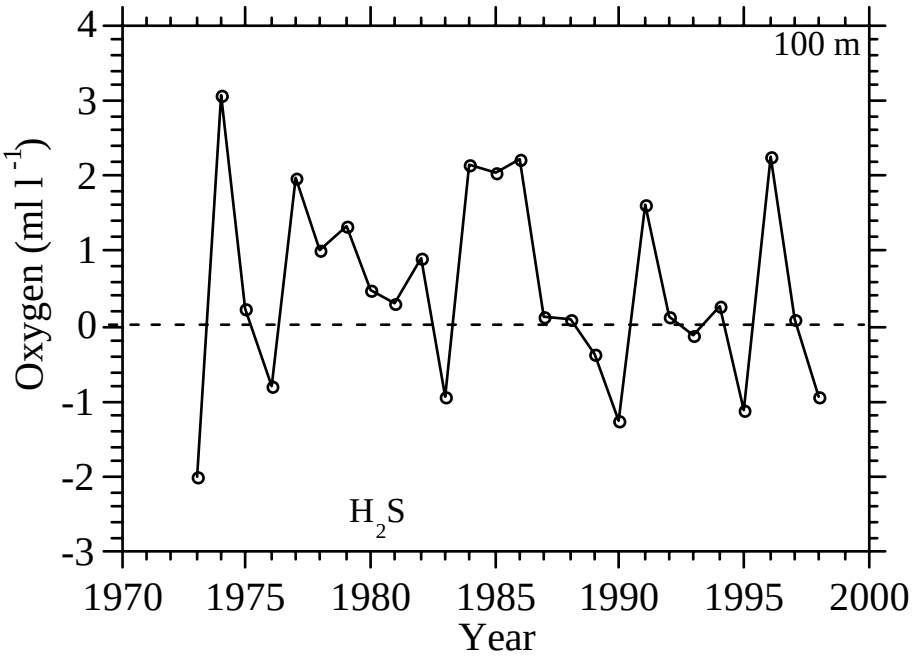

Figure 12. Oxygen concentration at 100 m in October during the years 1973 - 1998. Values from the Bunnefjord (EP1). Hydrogen sulphide concentration is plotted as negative O_2 concentration, and the dotted line is zero O_2 concentration.

Table 5. Number of years having oxygen concentrations 0.2 and 1.0 ml l^{-1} at 80, 100 and 125 m in October during the years 1973 - 1998 at the station in the Bunnefjord.

O_2 concentration	**Years at 80 m**	**Years at 100 m**	**Years at 125 m**
0.2	12	13	13
1.0	14	18	19

We do not have any data on denitrification in the sediment. Most denitrification measurements have been conducted in sediments (Seitzinger 1988; Fenchel et al. 1998) . Estimates from the Baltic indicate that denitrification in oxic-anoxic interfaces of the water column may equal sediment denitrification (Brettar and Rheinheimer 1991) . Denitrification in the sediment depends on NO_3 from the overlaying water or from NO_3 produced by nitrification in the sediment (Fenchel et al. 1998) . Denitrification should therefore be very low in the deep sediments underlaying the anoxic water masses in the Bunnefjord (no O_2, NO_3 or NO_2). During autumn 1998 we found high denitrification rates in the oxic-anoxic interface in the water column. In addition high rates probably occurred in sediments in shallow waters (depth less than about 80 m).

3.3.6 Denitrification in H_2S containing deep water?

Reduction of NO_3 to N_2 requires electron donors, usually dissolved organic C compounds. Some bacteria may also utilize reduced sulphur compounds (Fenchel and Blackburn 1979). H_2S driven denitrification have interesting implications. It does not need dissolved organic C compounds which usually is derived from the photosynthesis, thus it is not directly coupled to the carbon flux. Using the acetylene blockage method Brettar and Rheinheimer (1991) found H_2S driven denitrification in an oxic-anoxic interface in the Central Baltic.

We did not specifically measure H_2S but it was measured by backtitration with thiosulphate. It was noticeable from 82 m and below which is about 10 m below the water layer with high measured denitrification rates (Fig. 8). The increased potential denitrification rate below 78 m (Fig. 8) may have been H_2S driven. The ambient NO_3 and NO_2 concentrations were low (close to the detection limits) below 78 m, and the *in situ* rates were insignificant. In the fjord then, no evidence was found that denitrification was important in any part of the sulphide-bearing water mass.

3.3.7 Anammox (Anoxic ammonium oxidation)

The nutrient and O_2 profiles from 21 September open for the possibility that NH_4 may be oxidized by anaerobic bacteria to N_2 in the lower part of the NO_2 peak (75 - 80 m, Fig. 2). The reaction is known (Fenchel and Blackburn 1979), though it probably is more of an interesting possibility than a major nitrogen sink. Large surpluses of $^{15}NH_4$ and $^{14}NO_2$ were added to selected samples (Table 6). The surplus of $^{14}NO_2$ was added to ensure that a suitable electron acceptor was available. Significant amounts of labelled N_2 was produced in the samples from 74 - 80 m. The anammox rates were of the same order of magnitude as the denitrification rates at the same depths (Tables 5 and Appendix C). The anammox reaction was measured using unrealistic high concentrations of NH_4 and NO_2, and the rate is therefore not directly comparable with the denitrification rates. Consequently, anammox may have been significant locally in the watercolumn between 74 and 80 m, but it was of minor importance compared to the high denitrification rates at 66 - 74 m (Fig. 8).

Table 6. The anammox experiments. $^{14}N^{15}N$ excess, $^{15}N^{15}N$ excess (mmol N m^{-3}) and the anammox rate (mmol N m^{-3} d^{-1}).

Date/depth	$^{14}N^{15}N$ excess (mmol N m^{-3})	$^{15}N^{15}N$ excess (mmol N m^{-3})	Anammox (mmol N m^{-3} d^{-1})
25 September			
70	0.06	0.00	0.003
72	0.06	0.00	0.003
74	0.34	0.02	0.019
76	0.30	0.01	0.017
78	0.24	0.01	0.013
80	0.26	0.02	0.015
29 September			
74	0.17	0.01	0.010

4. Conclusions

Below 40 m, the concentration of NO_3 decreased with depth in the Bunnefjord and it was depleted in the anoxic deep water (below ca. 80 m). The concentrations of PO_4 and $Si(OH)_4$ increased with depth and were high in the anoxic deep water (below ca. 80 m). These differences in nutrient concentrations together with the very low total N : total P ratio (< 3) in the anoxic deep water clearly show that a major portion of the nitrogen is lost from the system. Denitrification is the most likely N sink in the fjord. A mass balance approach has previously been used to estimate N losses from anoxic basins, the Bunnefjord included, and denitrification has been suggested as a major N sink (literature data).

High NO_2 concentrations (maximum 4.39 mmol m^{-3}) were frequently observed at the oxic-anoxic interface. Such a subsurface NO_2 peak may have different origins, but being located at the oxic-anoxic interface in water with lowered NO_3 concentrations and very low NH_4 concentrations, it was probably present as a metastable intermediate in the denitrification process.

The used ^{15}N-technique (the isotope pairing method) proved to be a convenient method for measuring denitrification in the fjord. It is, however, time consuming and it requires sophisticated analytical procedures (mass spectrometry).

No significant denitrification was found in the surface (4 m) with high O_2 concentrations. The rest of the denitrification experiments were conducted in O_2-poor water sampled from 66-100 m depth. Usually, more than 90 % of the added $^{15}NO_3$ was recovered as labelled N_2 after 10 - 20 days. Denitrification was therefore the dominating process transforming N in our samples.

A profound lag phase (4 - 10 days) occurred in all the samples from the O_2-poor water mass. The lag phase was reduced to 4 days in the additional experiment in December which is comparable with literature values from the water column (Appendix D). The lag phase is suggested to occur because some intermediate(s) accumulated early during the incubation period. The NO_2 peak in the oxic-anoxic interface shows that NO_2 accumulated during denitrification in the Bunnefjord.

The potential denitrification rate which includes denitrification of added $^{15}NO_3$ was high in most of the O_2-poor water column, and maximum potential rate was 2.1 mmol m^{-3} d^{-1} at 70 m. A local minimum in the potential rate occurred in the lower part of the oxic-anoxic interface (76 - 78 m), and the potential rate increased again in the H_2S-containing deep water. This increase may have been H_2S-driven.

High *in situ* rates were restricted to the oxic-anoxic interface of the water column. The maximum *in situ* rate was 1.5 mmol m^{-3} d^{-1} at 70m. It was a significant linear correlation between the *in situ* denitrification rate and the ambient NO_3 concentration, and the *in situ* rate at low O_2 concentrations in the oxic-anoxic interface was therefore regulated by available NO_3.

Unfortunately denitrification was not measured between 4 and 66 m and we thereby probably missed the upper part of the denitrification peak. By using a combination of measured and modelled rates, integrated *in situ* denitrification rate was estimated to 13.4 mmol m^{-2} d^{-1}. It is also suggested that denitrification was confined to the deep density gradient around 70 m in the oxic-anoxic interface. Integrated *in situ* denitrification rate in this gradient (63 - 72 m) was 12.4 mmol m^{-2} d^{-1}. This will be crucial for any estimate of denitrification as N sink in the system, and it therefore needs to be verified.

The downward transport of nutrients and O_2 were obviously restricted by the density gradients. Reduced downward transport of O_2 and NO_3 into the O_2-poor deep water will eventually have opposite effects on the denitrification rate. Reduced O_2 influx may facilitate denitrification while reduced NO_3

influx may reduce denitrification. The vertical extension of denitrification needs to be confirmed before the effect of downward transport of O_2 and NO_3 can be evaluated.

Anoxic ammonium oxidation may have been significant locally but it was only a minor nitrogen sink in the water column.

Our main conclusions are that the denitrification rates in the oxic-anoxic interface in the Bunnefjord were very high and that denitrification must be a major N sink in the fjord.

5. Future research

The experiments described here are the first direct measurements of denitrification in Norwegian fjords. Most denitrification experiments, especially in the Nordic countries, have been conducted in sediments. Norwegian fjords are rare locations and knowledge from other marine systems will therefore not always apply. The fjords are deep and often have restricted circulation which may lead to anoxic conditions in the deep water. It is therefore reasonable to assume that denitrification in oxic-anoxic interfaces in the water column will be important in several fjords. This investigation has definitely increased our knowledge of denitrification in fjords like the Bunnefjord.

Denitrification is an important N sink in the coastal zone, especially in areas exposed to anthropogenic eutrophication. However, additional research is necessary before comparative studies of denitrification in different coastal environments can be made. Suggestions for additional research are given below. The additional research should preferably be conducted at the same station in the Bunnefjord:

- **Lag phase.** Time series experiments to study the lag phase. Additional experiments are needed to see what occurs during the lag phase.
- **Effects of O_2.** Measurements if denitrification in samples with different O_2 concentrations, natural samples with variable O_2 concentration and natural samples added O_2.
- **Effects of dissolved organic C.** Measurements of dissolved organic C in the samples combined with measurements of denitrification in natural samples spiked with organic C.
- **Depth profiles.** Detailed profiles of denitrification to check if denitrification is restricted to the deep density gradient.
- **Comparative studies in sediments and in the water column.** Direct comparison of denitrification in the water column and in the sediments in the same area.
- Studies to investigate seasonal variations and variation between different fjords.
- Experimental studies to investigate factors controlling denitrification in sediments and water column.

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Appendix A. Nutrients and oxygen

Nutrients and oxygen at Bunnefjorden (EP1) September - October 1998

Depth	Profile1 21.sep NO2	Profile1 21.sep NO3	Profile1 21.sep NH4	Profile1 21.sep O2	Profile2 21.sep NO2	Profile2 21.sep NO3	Profile2 21.sep NH4	Profile2 21.sep O2	Profile3 25.sep NO2	Profile3 25.sep NO3	Profile3 25.sep NH4	Profile3 25.sep O2	Profile4 25.sep NO2	Profile4 25.sep NO3	Profile4 25.sep NH4	Profile4 25.sep O2	Profile5 29.sep NO2	Profile5 29.sep NO3	Profile5 29.sep NH4
(m)	(µM)	(µM)	(µM)	(ml/l)	(µM)	(µM)	(µM)	(ml/l)	(µM)	(µM)	(µM)	(ml/l)	(µM)	(µM)	(µM)	(ml/l)	(µM)	(µM)	(µM)
0	0.46	6.1	2.6	5.83															
4	0.42		5	5.12					0.4	4.1	0.8	6.21							
8	0.38		7.1	4.4															
12	0.42		8.2	3.42															
16	0.11		<0.1	2.21															
20	0.11	18.5	<0.1	2.33															
25	0.07		<0.1	1.73															
30	0.1		<0.1	1.52															
40	0.06	26.4	<0.1	0.49															
45					0.16		1	1.7											
50	<0.05		<0.1	0.71															
60	0.05		<0.1	0.91	0.05	16	<0.1												
62					0.05	15.7	<0.1	0.83											
64					0.08	14.9	<0.1	0.48					0.08	15.3	0.1	0.72			
66					0.07	14.6	0.2	0.47	0.12	14.2	0.1	0.86	0.13	14.2	<0.1	0.42			
68					0.08	13.1	<0.1	0.46	0.06	13.2	<0.1	0.45	0.07	12.4	0.1	0.19	0.06		
69																	0.06		<0.1
70	3.47		<0.1	0.18	0.38	11.8	<0.1	0.18	0.05	11.2	0.1	0.34	0.55	9.1	0.1	0.13	0.06		<0.1
71																	0.59		
72					3.87	6.1	<0.1	0.17	1.02	6.7	0.2	0.2	1.21	6.4	<0.1	0.11	3.51		<0.1
73																	3.95		<0.1
74					4.39	0.5	<0.1	0.15	1.57	1.6	<0.1	0.23	1.22	6	0.1	0.11	1.96		<0.1
75																	1.97		<0.1
76					1.13	<0.2	0.1	0.15	1.13	<0.2	0.1	0.24	1.65	1.8	0.1	0.17	0.93		<0.1
77																	0.08		0.3
78					0.12	<0.2	0.5	0.15	<0.05	<0.2	0.2	0.25	<0.05	<0.2	0.3	0.15	0.21		0.2
80	<0.05		1.9	0.08	0.06	<0.2	1.2	0.1					0.09	<0.2	2.3	0.11			
82					0.07	0.3	2.7	0.07	0.05	<0.2	2	0.28	0.07	<0.2	2.7	0.11			
84					0.08	0.3	3.5	0.06											
86					0.06	0.3	3.6	0.06	<0.05	<0.2	3.3	0.28	0.1	<0.2	3.1	0.08			
88					0.07	0.3	3.5	0.05											
90	<0.05		3.5	-0.16	0.14		3.5	-0.16											
100	<0.05		4	-0.27					<0.05	<0.2	3.8	-0.05							
110	<0.05		4.2	-0.33															
125	<0.05		4.2	-0.3															
140	0.07		5.3	-0.07															
150	<0.05		8.2	-0.48															

n ml/l.

Tab. cont.

Depth	Profile6 29.sep NO2	Profile6 29.sep NO3	Profile6 29.sep NH4	Profile6 29.sep O2	Profile8 20-0ct NO2	Profile8 20-0ct NO3	Profile8 20-0ct NH4	Profile8 20-0ct O2	Profile9 20-0ct NO2	Profile9 20-0ct NO3	Profile9 20-0ct NH4	Profile9 20-0ct O2
(m)	(µM)	(µM)	(µM)	(ml/l)	(µM)	(µM)	(µM)	(ml/l)	(µM)	(µM)	(µM)	(ml/l)
0					0.56	4.4		6.58				
4					0.65	4.7		6				
8					0.64	6.9		4.52				
12					1.23	19.5		1.65				
16					0.46	19.5		1.9				
20					0.09	19.9		1.88				
25					0.06	20.4		1.56				
30					0.09	21.3		1.18				
40					0.08	26.4		0.39				
45												
50					0.05	18.5		0.55				
60					0.09	15.6		0.74				
62												
64												
66												
68									1.08			
69									1.69			
70	0.12	11.7	<0.1	0.25	3.04	4.6		0.12	1.12			
71									3.12			
72	1.54	8.1	<0.1	0.18					0.71			
73												
74	2.39	3.2	<0.1	0.32					0.99			
75									<0.05			
76									<0.05			
77									<0.05			
78									<0.05			
80					0.09	0.2		0.06				
82	0.1	0.3	0.1	0.18								
84												
86												
88												
90					0.06							
100					0.06	<0.2						
110						0.2						
125												
140												
150												

Appendix B. Incubation experiments

Time series denitrification from the Bunnefjord September - October 1998.
Relative 14N15N excess and relative 15N15N excess multiplied by N2 concentration (se methods)

Depth (m)	Days	14N15N (µM N)	15N15N (µM N)
25.sep			
4	1	0.01	0
4	2	0	0
4	4	0	0
4	11	0	0
4	20	0	0
66	1	0	0
66	2	0	0
66	4	0	0
66	11		
66	11		
66	11	2.25	0.36
66	20	3.66	0.55
70	1	0.01	0
70	2	0.01	0
70	4	0.01	0
70	4	0.01	0
70	4	0.01	0
70	11	2.92	0.55
70	20	2.9	0.55
72	1	0	0
72	2	0	0
72	4	0.01	0
72	11	1.34	0.35
72	11	2.83	0.8
72	11	1.64	0.46
72	20	1.77	0.46
74	1	0	0
74	2	0.01	0
74	4	0.01	0
74	11		
74	11	1.22	0.68
74	11	1.62	0.91
74	20	1.44	0.85
76	1	0.01	0
76	2	0.01	0
76	4	0	0
76	11	0.27	0.33
76	11	0.28	0.36
76	20	0.43	0.59
78	1	0.01	0
78	2	0	0
78	4	0	0
78	11		
78	11	0.09	0.46
78	11		
78	20	0.09	0.31
82	1	0	0
82	2	0	0
82	4	0	0
82	11	0.25	1.39
82	11	0.16	1
82	11	0.26	1.5
82	20	0.29	1.62
82	20	0.31	1.59
82	20	0.39	1.76

Depth (m)	Days	14N15N (µM N)	15N15N (µM N)
25.sep			
86	1	0.01	0
86	2	0.01	0
86	4	0.06	0.31
86	11	0.39	2.61
86	11	0.38	2.34
86	11	0.26	1.56
86	20	0.84	2.4
100	1	0.01	0
100	2	0	0
100	4	0.01	0.03
100	11	0.41	3.17
100	20	0.3	3.03
29.sep			
70	1	0.01	0
70	2	0	0
70	4	0	0
70	10	1.27	0.23
70	10		
70	20	3.43	0.58
72	1	0.01	0
72	2	0.01	0
72	4	0.01	0
72	10	2.03	0.42
72	10	2.34	0.49
72	10	2.19	0.45
72	20	3.36	0.66
74	1	0	0
74	2	0.01	0
74	4	0.01	0
74	10	1.06	0.39
74	10	1.25	0.46
74	20	1.76	0.56
82	1	0.01	0
82	2	0.01	0
82	4	0.01	0
82	10	0.15	1.12
82	10	0.13	1.06
82	20	0.45	2.52

Appendix C. Denitrification rates

Concentrations of nutrients and oxygen and denitrification rates from the Bunnefjord September - October 1998

Depth (m)	NO2 (µM)	NO3 (µM)	NH4 (µM)	O2 (ml/l)	Denitrification Potential (µM N/d)	Denitrification in situ (µM N/d)
25.sep						
4	0.4	4.1	0.8	6.21	0	0
66	0.12	14.2	0.11	0.86	1.75	1.33
70	0.05	11.2	0.1	0.34	2.1	1.52
72	1.02	6.7	0.2	0.2	0.94	0.61
74	1.57	1.6	0.08	0.23	0.82	0.39
76	1.13	<0.2	0.11	0.24	0.2	0.06
78	<0.05	0.2	0.17	0.25	0.16	0.01
82	0.05	0.2	2.01	0.28	0.44	0.03
86	<0.05	0.2	3.25	0.28	0.72	0.05
100	<0.05	0.2	3.75	-0.05	1.03	0.06
29.sep						
4						
66						
70	0.12	11.7	<0.05	0.25	1.08	0.8
72	1.54	8.1	0.05	0.18	1.76	1.24
74	2.39	3.2	<0.05	0.32	0.72	0.42
76						
78						
82	0.1	0.3	0.11	0.18	0.42	0.03
86						
100						

Appendix D. Lag phase experiment

Introduction

One possible explanation of the lag phase (see section 3.3.1) is that NO_3 inhibits the reduction of NO_2 to N_2, and NO_2 therefore accumulates when NO_3 is available. We did a first lag phase experiment in December and asked the following questions:

- Will elevated NO_3 concentrations affect reduction of NO_2 to N_2?
- Will O_2 additions to the incubation tubes affect reduction of NO_3 to N_2?

We did 5 series of incubations to study the influence of elevated NO_3 concentrations on the reduction of NO_3 or NO_2 to N_2 (the NO_3/NO_2-experiment) and to study the influence of O_2 additions on the reduction of NO_3 to N_2 (the O_2-experiment; Table D1).

Materials and methods

Two NO_3-poor samples were collected at 72 and 86 m at the same station in the Bunnefjord (EP1) on 15 December 1998. The depths were selected based on the nutrient profiles from the cruises in September and October and the hydrography from all the cruises. The 72 m sample was collected immediately below the NO_2 peak and the 86 m sample was from H_2S containing water (H_2S odour). Later measurements showed that the concentrations of NO_2 and NO_3 indeed were low in the 2 samples (Fig. D1), and so was the O_2 concentration (< 0.1 ml l^{-1}). The NO_3/NO_2-experiment was conducted as described above (section 2.2) with 2 exceptions. Incubation time was shorter for most of the subsamples (usually 1 - 8 days) and the isotope was added as $^{15}NO_3$ (series 1 and 4) or $^{15}NO_2$ (series 3 and 5) to separate tubes (same concentration in each tube). Only water from 72 m was used in the O_2-experiment. Series 1 served as a control and series 2 was treated identically except that 3 ml O_2 l^{-1} was added to the incubation tubes at day 4 of the incubation.

Results and discussion

The NO_3/NO_2-experiment (Series 1, 3, 4 and 5)

The samples (NO_3-poor) were incubated with $^{15}NO_3$ and $^{15}NO_2$. If NO_3 inhibits reduction of NO_2 to N_2, no significant lag phase should be found in the samples incubated with $^{15}NO_2$. The samples incubated with $^{15}NO_3$ served as a control and a profound lag phase was expected in these samples.

Significant single and double labelled N_2 ($^{14}N^{15}N$ excess and $^{15}N^{15}N$ excess respectively) were found in samples from both depths irrespectively of $^{15}NO_3$ or $^{15}NO_2$ were added (Figs. D2 and D3). A profound lag phase of about 4 days was found in the samples from 86 m added $^{15}NO_3$ or added $^{15}NO_2$ (Fig. D2). The values from 72 m were all very low and increased slightly after 4 days (Fig. D3). All the samples were collected in NO_3-poor water, and the corresponding *in situ* denitrification rates from 72 and 86 m were both very low (Table D2). Similar low *in situ* rates were found also in NO_3-poor water in September - October (see section 3.2.2 and Fig. 8), thus the rates in Table D2 are not surprisingly low. The growth conditions at 86 m with H_2S present will be different from the conditions offered at 72 m (no H_2S). Different populations will probably also dominate at the two depths because of the H_2S. The results from the experiment from 86 m will therefore not necessarily apply for samples collected in the denitrification peak around 70 m. Unfortunately the values from 72 m are low and doubtful, though the rates indicate a 4 days lag phase independent of ambient NO_3.

The O_2-experiment (Series 1 and 2)

No significant rates were found in samples added O_2 (Tables D2 and D4). It is well known denitrification only occurs under low O_2 concentrations (see section 3). In section 6.3 we argued that O_2 did not cause the lag phase. We did the O_2-experiment to see if denitrification would stop in the incubation tubes if O_2 was added directly to the tubes, and 3 ml l^{-1} of O_2 did stop the denitrification.

NO_3 concentrations in September - December

Values from September - October were also presented above (Fig. 11). The NO_3 concentration at 70 m decreased significantly with time. It decreased from about 11 mmol m^{-3} during the 3 cruises in September to 2.2 mmol m^{-3} in December (Fig. D4). The denitrification rates were high around 70 m in September (Fig. 8), and it is reasonable to assume that denitrification was the major NO_3 sink. Denitrification was not measured in October and it was only measured in NO_3-poor water in December (72 and 86 m). However, the high NO_2 concentrations around 70 m (>1.7 mmol m^{-3}; Fig. 3 and Fig. D1) indicate high denitrification rates during the whole period September - December.

Conclusions of the lag phase experiment

NO_3 did not affect the reduction of NO_2 to N_2 in H_2S containing water at 86 m. A profound lag phase of 4-5 days was found irrespective of NO_3 was present or not. The values from H_2S-free water (72 m) are very low and the importance of NO_3 is less clear. However, the values indicate that NO_3 did not affect the reduction of NO_2 to N_2 in H_2S-free water either.

O_2-additions stopped denitrification completely for 11 days or longer. However, we still believe that O_2 did not cause the lag phase (see section 3.3.1).

Future lag phase experiments

Our experimental design was very simple of two reasons. This was our first lag phase experiment and we did not expect a pronounced lag phase in the samples incubated with $^{15}NO_2$. The reduction of NO_3 to N_2 is a multistep reaction also involving the intermediates NO and N_2O. The lag phase is therefore complex and more detailed experiments are needed. Future experiments should include:

Accumulation of the intermediates. We plan to have some of the samples from December analyzed for N_2O (gas chromatography). The next step will be to see if any of the other intermediates accumulate during the lag phase.
Decrease in NO_2 or NO_3. The denitrification rates from some of the samples were high and it should be possible to measure decrease in concentration of added NO_3 or NO_2.
O_2 concentration in the tubes. Unintended O_2 additions to the incubation tubes during filling or leakage during incubation will affect denitrification. O_2 should therefore be checked in the incubation tubes.

Table D1. Series of incubations in the NO_3/NO_2- and O_2-experiments. Sampling depths and additions of $^{15}NO_3$, $^{15}NO_2$ and O_2 are given.

Series	Depth	Additions	Experiments
1	72	$^{15}NO_3$	NO_3/NO_2- and O_2-experiments
2	72	$^{15}NO_3+O_2$	O_2-experiment
3	72	$^{15}NO_2$	NO_3/NO_2-experiment
4	86	$^{15}NO_3$	NO_3/NO_2-experiment
5	86	$^{15}NO_2$	NO_3/NO_2-experiment

Table D2. *In situ* and potential denitrification rates[i] (mmol N m^{-3} d^{-1}) in the samples from 72 and 86 m collected 15 December. Rates are also given for samples from 72 m added O_2. Average ± range are given (n=2).

Depth	*In situ* denitrification rate	Potential denitrification rate
72	0.01±0.01	0.10±0.03
86	0.03±0.00	0.51±0.00
$72+O_2$	<0.005	<0.005

[i]The rates are calculated from samples incubated 10 days and assuming a constant 4 days long lag phase, see section 2.2.

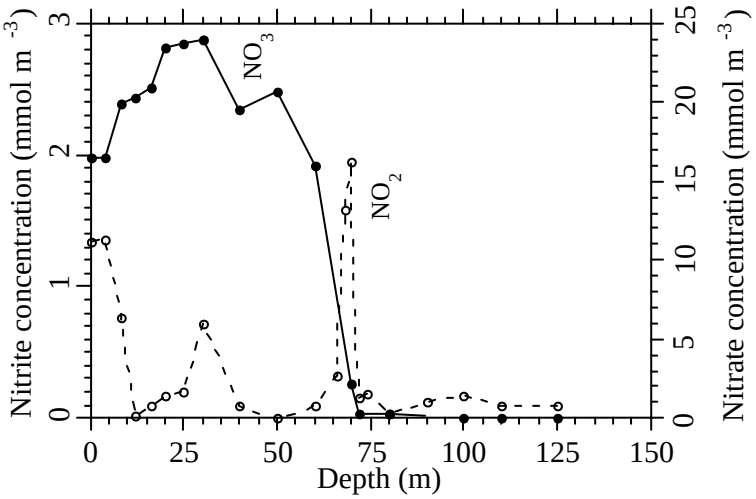

Figure D1. Depth profiles of NO_2 and NO_3 (mmol m^{-3}) on 15 December 1999

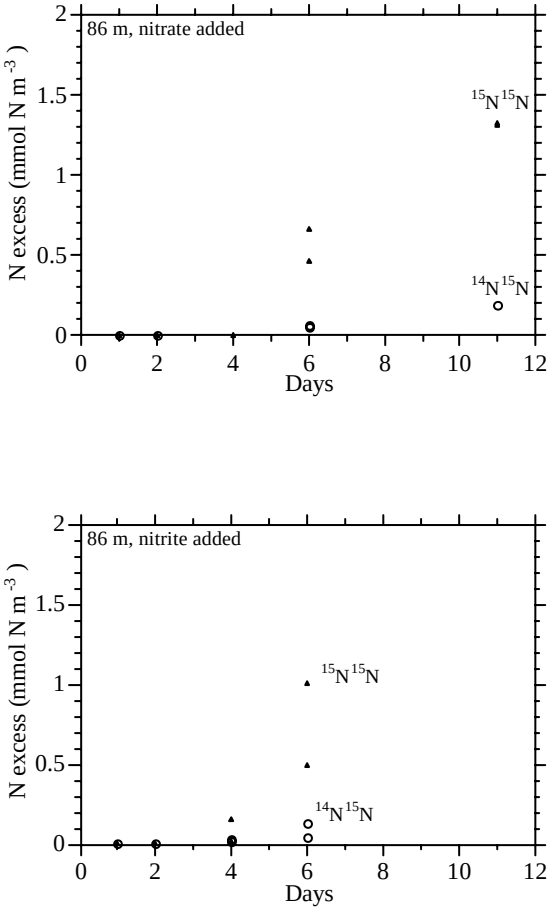

Figure D2. $^{14}N^{15}N$ excess and $^{15}N^{15}N$ excess in mmol N m^{-3} plotted against incubation times in days in samples from 86 m on 15 December. ^{15}N added as $^{15}NO_3$ (top figure) or $^{15}NO_2$ (bottom figure). Ambient NO_3 and NO_2 concentrations were both <0.2 mmol m^{-3}.

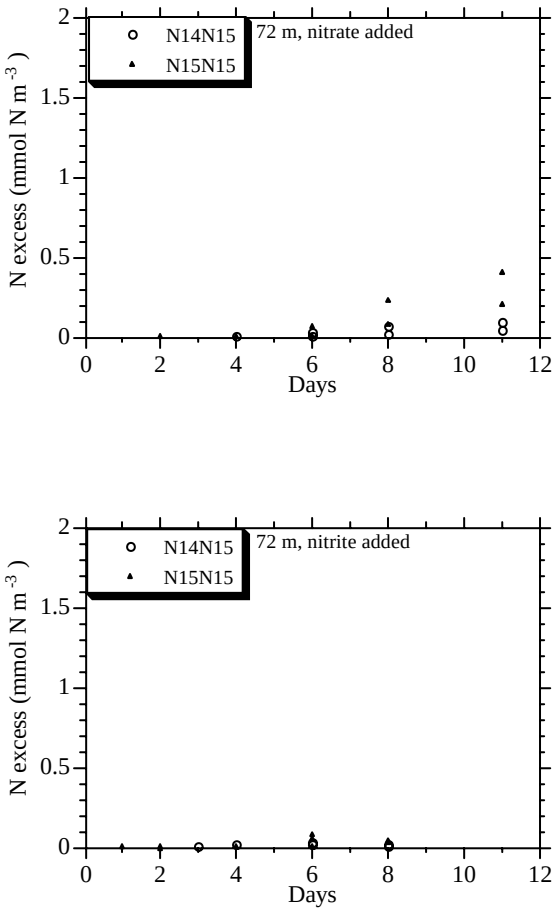

Figure D3. $^{14}N^{15}N$ excess and $^{15}N^{15}N$ excess in mmol N m^{-3} plotted against incubation times in days in samples from 72 m on 15 December. ^{15}N added as $^{15}NO_3$ (top figure) or $^{15}NO_2$ (bottom figure). Ambient concentration of NO_3 and NO_2 were 0.27 and 0.16 mmol m^{-3} respectively.

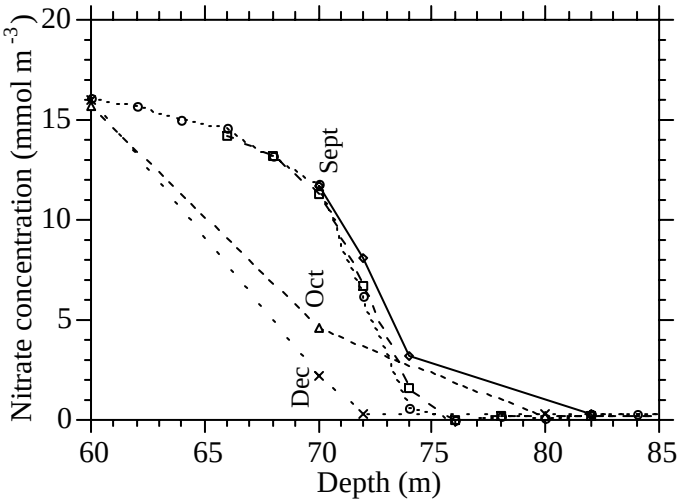

Figure D4. Depth profiles of NO_3 concentration (mmol m^{-3}) between 60 m and 85 m on 21, 25 and 29 September (circles, squares and diamonds respectively), 20 October (triangles) and 15 December (x's).

Tables D3 and D4 give concentrations and denitrification rates from the experiment started 15 December 1998.

Table D3. Concentrations of nutrients and oxygen and denitrification rates from the Bunnefjord 15 December 1998.

	Concentration			Denitrification rate	
Depth	NO_2	NO_3	O_2	Potential	In situ
(m)	(µM)	(µM)	(ml/l)	(µM N/d)	(µM N/d)
15 December					
0	1.34	16.5	6.69		
4	1.35	16.5	6.55		
8	0.76	20.0	4.07		
12	<0.05	20.3	2.38		
16	0.09	21.0	1.48		
20	0.17	23.4	1.17		
25	0.2	23.7	1.02		
30	0.72	23.9	0.68		
40	0.1	19.5	0.81		
50	<0.05	20.7	0.3		
60	0.09	16.0	0.57		
66	0.32				
68	1.58				
70	1.95	2.2	0.06		
72	0.16	.3	0.18	0.10	0.01
74	0.18				
80	<0.05	.3	-0.42		
86	<0.1	<0.2	<0	0.51	0.03
90	0.12		-0.46		
100	0.17	<0.2	-0.53		
110	0.1	<0.2	-0.48		
125	0.1	<0.2	-0.7		
140			-0.9		
150			-1.1		

Table D4. Time series of denitrification from the Bunnefjord 15 December 1998. Added ^{15}N compound and oxygen are indicated. Relative $^{14}N^{15}N$ excess and relative $^{15}N^{15}N$ excess are multiplied by N_2 concentration (see methods).

Depth	^{15}N added	Days	$^{14}N^{15}N$ (µM N)	$^{15}N^{15}N$ (µM N)
72	NO3	2	0.00	0.00
72	NO3	2	0.00	0.00
72	NO3	4	0.00	0.00
72	NO3	4	0.00	0.00
72	NO3	6	0.03	0.07
72	NO3	6	0.00	0.01
72	NO3	8	0.02	0.08
72	NO3	8	0.07	0.23
72	NO3	11	0.10	0.41
72	NO3	11	0.05	0.20
72	NO2	1	0.00	0.00
72	NO2	1	0.00	0.00
72	NO2	2	0.00	0.00
72	NO2	2	0.00	0.00
72	NO2	2	0.00	0.00
72	NO2	3	0.00	0.00
72	NO2	4	0.01	0.00
72	NO2	4	0.02	0.00
72	NO2	4	0.02	0.00
72	NO2	6	0.02	0.01
72	NO2	6	0.02	0.08
72	NO2	6	0.03	0.06
72	NO2	8	0.02	0.04
72	NO2	8	0.01	0.04
86	NO3	1	0.00	0.00
86	NO3	1	0.00	0.00
86	NO3	2	0.00	0.00
86	NO3	2	0.00	0.00
86	NO3	4	0.00	0.00
86	NO3	4	0.00	0.00
86	NO3	6	0.07	0.66
86	NO3	6	0.05	0.47
86	NO3	11	0.19	1.32
86	NO3	11	0.19	1.33
86	NO2	1	0.01	0.00
86	NO2	1	0.00	0.00
86	NO2	2	0.00	0.00
86	NO2	2	0.00	0.00
86	NO2	2	0.01	0.00
86	NO2	4	0.01	0.02
86	NO2	4	0.03	0.16
86	NO2	6	0.14	1.01
86	NO2	6	0.04	0.49
Samples added oxygen				
72	NO3+O2	4	0.00	0.00
72	NO3+O2	4	0.00	0.00
72	NO3+O2	6	0.00	0.00
72	NO3+O2	6	0.00	0.00
72	NO3+O2	11	0.00	0.00
72	NO3+O2	11	0.00	0.00

Appendix E. Methods (in Norwegian)

Metodebeskrivelse for måling av denitrifisering og anammox

Denitrifiseringseksperimenter

Rørene fylles med prøve og tilsettes $^{15}NO_3$. Viktig at prøvene ikke "forurenses" med O_2 eller N_2 fra luften.

1. Fyll rørene helt fulle med topp uten luftblærer. Rørene fylles som O_2 flasker (slange uten luftblærer holdes i en liten bue opp og ned i bunnen av røret, prøven skal flomme over 2-3 ganger flaskevolumet). Gå raskt til punkt 2.

   Rørene er danske (dansk KEBO nr. 107.201-5) med skrukork og septum. Selges ikke hos KEBO i Norge, og må bestilles spesielt. Rørene er merket 5-ml, men det faktiske volumet er 6,7 ml. Type kork er viktig, flere er giftige. De danske skal være bra. Større volum hjelper ikke på følsomheten fordi det er mye N_2 i prøven. Må eventuelt bytte ut N_2 med en annen gass som ikke påvirker analysen. Større volum vil gi mer representative prøver, men må da inkubere underprøver av en større homogen prøve. En annen mulighet er å inkubere større volum og ta ut en mindre mengde (ca. 5 ml) for analyse.

2. Tilsett 25 µl av 1,0 mM $^{15}NO_3$ med stempelpipette i bunnen av røret og skru korken raskt på. Sjekk for luftblærer ved å snu røret på hodet og riste eller dunke på røret. Konsentrasjonen i røret av tilsatt $^{15}NO_3$ blir 4 mmol m^{-3}.

   Volum < 50 µl kan tilsettes med sprøyte gjennom korken. Kan eventuelt ta av korken for å sette til større volum, f eks 100 µl iflg Aarhus. Valgte å sette til isotopen før korken ble skrudd på fordi jeg ikke hadde nøyaktige sprøyter. Isotopen var KNO_3 med 99 at-% ^{15}N. Laget først en løsning på 10,0 µmol ml^{-1} i milli-Q (ionebyttet dest vann) og tynnet denne til 1,0 µmol ml^{-1} med kunstig sjøvann (3 % NaCl i milli-Q) rett før bruk. Brukte kunstig sjøvann for å hindre at isotopen flyter opp. Har ikke sjekket hvor mye isotop som flyter opp. Det er imidlertid lett å se at 25 µl rent milli-Q vann flyter opp samtidig som det blandes litt med sjøvannsprøven.

   Utregnet tilsetning: 0.025ml*1,0µmol ml^{-1}*1000/6,7=3,7 mmol m^{-3}.

3. Prøvene inkuberes mørkt ved *in situ* temperatur (15 ^oC for 4 meter prøven og 7 ^oC for de øvrige prøvene).

4. Prøvene tas ut etter 1, 2, 4, 10 og 20 døgn og fikseres med 50 µl formalin (ca 37 %). Sett en liten kanyle gjennom korken og tilsett formalin gjennom en 1-ml sprøyte med liten kanyle som også stikkes gjennom korken. Pass på at det ikke kommer inn luftblærer. Ta raskt ut kanylene og stram til korken. Prøvene kan oppbevares ved romtemperatur etter fiksering. Nitrogen i vanlig luft består av 99,6 % ^{14}N og nitrogen fra luften vil derfor forurense prøvene.

Anammox-eksperimenter

Rørene fylles med prøve og tilsettes $^{15}NH_4$ og $^{14}NO_2$. Viktig at prøvene ikke "forurenses" med oksygen eller nitrogen fra luften. Alle anammox-prøvene ble tilsatt ammonium og nitritt med kanyler gjennom korken dagen etter innsamling.

1. Fyll rørene helt fulle med topp uten luftblærer og skru igjen korken. Rørene fylles som oksygenflasker (slange uten luftblærer holdes i en liten bue opp og ned i bunnen av flasken, prøven skal flomme over 2-3 ganger flaskevolumet). Sjekk for luftblærer ved å snu røret på hodet og riste eller dunke på røret.

   Rørene er danske (dansk KEBO nr. 107.201-5) med skrukork og septum. Selges ikke hos KEBO i Norge, og må bestilles spesielt. Rørene er merket 5-ml, men faktisk volum er 6,7 ml. Type kork er viktig, flere er giftige. De danske skal være bra.

2. Tilsett 50 µl av 5,0 mM $^{15}NH_4$ og 50 µl av 1,0 mM $^{14}NO_2$ med 1-ml sprøyter med små kanyler gjennom korken. Sett først inn en liten kanyle for å slippe ut overskudd. Pass på at det ikke kommer inn luftblærer. Konsentrasjonen i røret av tilsatt $^{15}NH_4$ skal være 37 µM og av tilsatt $^{14}NO_2$ skal være 7 µM.

   Utregnet tilsetning av $^{15}NH_4$: 0.050ml*5,0µmol ml^{-1}*1000/6.7=37,3 mmol m^{-3} .
   Utregnet tilsetning av: $^{14}NO_2$: 0.050ml*1,0µmol ml^{-1}*1000/5=7,4 mmol m^{-3} .

3. Prøvene inkuberes mørkt ved *in situ* temperatur (7 ^{o}C).

4. Prøvene tas ut etter 20 døgn og fikseres med 50 µl formalin (37 % formaldehydløsning). Sett en liten kanyle gjennom korken og tilsett formalin gjennom en 1-ml sprøyte med liten kanyle som også stikkes gjennom korken. Pass på at det ikke kommer inn luftblærer. Ta raskt ut kanylene og stram til korken. Prøvene kan oppbevares ved romtemperatur etter fiksering.

Måling av ^{15}N

Prøvene ble tilsatt helium og ^{15}N i helium ble målt i et massepektrometer (MS) ved Universitetet i Aarhus. Vi målte 10 - 15 prøver på en time. Utstyret trengte ofte mindre justeringer og vi kunne som regel måle sammenhengende i 2 - 4 timer (kalt måleomgang under). Vi brukte 7 måleomganger på å måle totalt 336 prøver og standarder.

1. Til hver prøve tilsettes 1-ml helium med en kanyle gjennom korken. Sett først inn en kanyle med tom sprøyte og deretter en kanyle med sprøyte med 1 ml helium. Når heliumen sprøytes inn vil tilsvarende mengde prøve presses inn i den tomme sprøyten. Ta raskt ut begge kanylene og ryst prøven kraftig i 1/2-1 minutt. Rystingen er veldig viktig. Det er fortsatt viktig å ikke forurense prøvene med luft. Sprøyten fylles med helium ved å stikke kanylen inn i en heliumstrøm og forsiktig dra inn 1 ml.

2. MS klargjøres og standarder måles. Atmosfærisk luft ble brukt som standard og til korreksjon for naturlig bakgrunn. Mengde standard ble tilpasset nitrogenmengden i prøvene (totalt areal fra standarden ble tilpasset totalt areal fra prøvene). Vanligvis ble det brukt 20 µl luft, noen få ganger 25 µl. Drift er et stort problem i MS som ble brukt. Vi målte derfor 2-3 standarder etter hver tredje prøve.

3. 250 µl prøve (helium med ^{15}N) tas ut av et rør og sprøytes inn i MS.

4. Totalarealet må sjekkes slik at totalarealet for standard og prøve er omtrent likt. Hvis totalarealet for prøven er vesentlig lavere enn totalarealet for standarden så er noe av prøven tapt. Hvis totalarealet for prøven er vesentlig høyere enn totalarealet for standarden så er prøven forurenset med nitrogen fra luften. En prøve med vesentlig lavere totalareal må måles på nytt. En prøve med vesentlig høyere totalareal kan "reddes" ved å justere i utregningen for nitrogen tilført fra luften. I de resultatene som presenteres her ble alle prøvene med avvikende totalareal målt på nytt.

5. Ny prøve måles, standarder etter hver tredje prøve.

Beregning av $^{14}N^{15}N$ og $^{15}N^{15}N$

1. Regn ut relativ $^{14}N^{15}N$ (=area $^{14}N^{15}N$/(area$^{14}N^{14}N$ + area$^{14}N^{15}N$ + area$^{15}N^{15}N$) og relativ $^{15}N^{15}N$ (=area $^{15}N^{15}N$/(area$^{14}N^{14}N$ + area$^{14}N^{15}N$ + area$^{15}N^{15}N$) for standard (=bakgrunn) og for prøvene.

2. Regn ut $^{14}N^{15}N$ excess og $^{15}N^{15}N$ excess for prøvene ved å trekke fra tilhørende bakgrunnsverdiene. Avlesningene må imidlertid først sjekkes for instrumentdrift. Det lønner seg å behandle hver måleomgang for seg (se måling av ^{15}N over). Prøver og standarder nummereres fortløpende av MS i hver måleomgang (= injeksjonsnr.). Vi korrigerte for instrumentdrift ved å kjøre lineær regresjonsanalyse mellom relativ $^{14}N^{15}N$ (eller $^{15}N^{15}N$) og injeksjonsnummer for standardene. Bakgrunnskorreksjonene for de enkelte prøvene kan da regnes ut fra formelen for regresjonslinjene og injeksjonsnummer for prøvene. I de resultatene som presenteres her var det instrumentdrift for $^{14}N^{15}N$ i 4 av de 6 måleomgangene og i 1 av de 6 måleomgangene for $^{15}N^{15}N$.

Beregning av denitrifiseringshastighetene

Utregningene er gjort ifølge Nielsen (1992). Mengde N_2 er regnet ut fra temperatur og saltholdighet (Riley and Skirrow 1975). For de aktuelle prøvene var N_2 konsentrasjonen 1.1 mol N m^{-3}. Denitrifiseringen av $^{15}NO_3$ (=D_{15}) og $^{14}NO_3$ (=D_{14}) beregnes fra de målte produksjonene av $^{14}N^{15}N$ (=P_{29}) og $^{15}N^{15}N$ (=P_{30}):

P_{29} = excess$^{14}N^{15}N$*(1.1 mol N m^{-3})
P_{30} = excess$^{15}N^{15}N$*(1.1 mol N m^{-3})
$D_{15} = P_{29} + 2*P_{30}$
$D_{14} = D_{15} *(P_{29}/(2*P_{30}))$.

Nitratisotopen ble tilsatt som 99 at-% $^{15}NO_3$. Tilsatt $^{14}NO_3$ var dermed bare 0.04 mmol m^{-3} og ble sett bort fra i utregningene. D_{14} er dermed *in situ* denitrifiseringsrate og $D_{14} + D_{15}$ er potensiell denitrifiseringsrate.

Beregning av anammox-hastigheten
Regnes ut som over, men anammox raten = D_{15}.